AD-A143 916 THE EFFECT OF A LOW FLUORIDE DELIVERY SYSTEM ON BACTERIAL METABOLISM(U) CONNECTICUT UNIV HEALTH CENTER FARMINGTON N TINANOFF ET AL. 25 AUG 81 1/1

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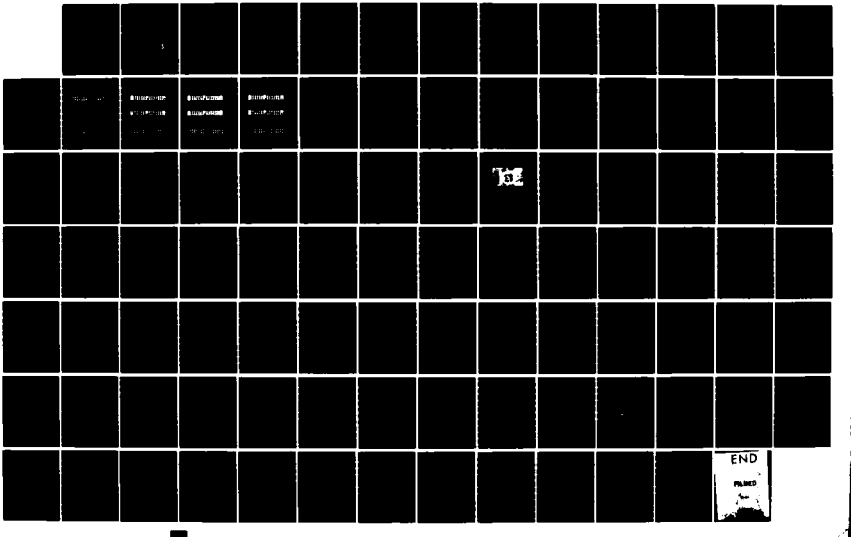

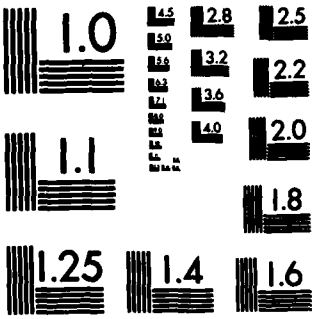

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

THE EFFECT OF A LOW FLUORIDE DELIVERY SYSTEM ON BACTERIAL METABOLISM

Annual Report

By

Norman Tinanoff, D.D.S., M.S.

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August 25, 1981

(For period 1 September 1980 to 30 June 1981)

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University of Connecticut Health Center

Farmington, Connecticut 06032

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stannous flouride

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The purpose of this contract is to develop and test agents and delivery systems which can prevent and control emergencies in field situations where dental care may be impossible. In this report, three areas are discussed: (1) initiation of a clinical trial to test the disease reducing potential of SnF_2; (2) development and clinical testing of a controlled release delivery system for SnF_2 to impart preventive effects without necessity for soldier cooperation; and (3) *in vitro* trials to find the optimum concentration and conditions of SnF_2 for disease control as well as exploring other possible agents for these properties.

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SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

The clinical trial to test the disease reducing potential of SnF_2 is now underway with 34 patients, 6 of which have had their 6 month examinations. The patients have been systematically divided into a NaF and a SnF_2 group. Some patients who are not interested in being in the study but don't mind the examinations will constitute a third group. After one, three and six months, saliva and plaque samples are analyzed and several dental health parameters are recorded. The data from the six month exams is presently being tabulated and analyzed. Several oral presentations of this data are planned to be ready by October, 1981.

Pilot studies examining the physical and clinical properties of an intracoronal controlled release fluoride delivery system were performed. After testing various percentages of SnF_2 incorporated into polycarboxylate, zinc phosphate, IRM, and zinc oxide eugenol cements, 70 percent SnF_2 in polycarboxylate cement was found to have adequate compressive strength while releasing the greatest amount of fluoride in vitro.

A 30-day in vivo trial in which this fluoride-cement was used as a temporary intracoronal restoration produced elevated salivary fluoride levels with only transient elevation in urinary fluoride levels. Plaque scores decreased during the experimental period suggesting that the released SnF_2 affected bacterial growth or attachment. The SnF_2-polycarboxylate cement was an adequate temporary restorative material without significant side effects.

The in vitro trials to test the optimum concentration and conditions for antiplaque properties have found that SnF_2 is the only cationic agent tested which has antiplaque properties. Other agents tested have been sodium fluoride, stannic fluoride, zinc fluoride, lead fluoride, stannous chloride, zinc chloride and lead chloride. The most likely reason for this finding is that only SnF_2 concentrates within the bacteria cells as identified by electron microscopy and atomic absorption spectrophotometry. Other agents (e.g., PbF_2) accumulate on bacterial cell walls but do not have disease reducing potential; or do not accumulate in or on the bacteria at all (e.g., NaF, $SnCl_2$, $ZnCl_2$, ZnF_2, SnF_4). The optimum pH for SnF_2 is 3.0 and all antibacterial properties are lost above 4.0 when the agent appears to dissociate.

TABLE OF CONTENTS

Summary

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Two-Year Clinical Trial Using SnF_2

Introduction

In short-term studies, we have previously shown that mouthrinsing with SnF_2 inhibits bacterial accumulation on teeth and a regimen such as this might be appropriate in Army field situations where customary mechanical oral hygiene procedures may not adequately be performed. Prior to implementing such a preventive modality in soldiers, another clinical trial is necessary to examine long-term efficacy as well as possible side effects of this mouthrinse regimen.

The first clinical trial was conducted for only 2 weeks on dental students who were essentially in ideal oral health. The population that we have identified in this study are new clinic patients, 15-55 years old, who exhibit very poor oral hygiene as well as severe dental diseases.

Materials and Methods

1. Saliva samples from 60 patients, 15-55 years olds, in poor oral health was first collected. Patients harboring more than 200,000 Streptococcus mutans per ml saliva and rampant caries constituted the patient material in this study (Table 1).
2. At the second visit--Day 1 of the study--new saliva samples, as well as supra- and subgingival plaque, were collected from these patients. From the two samples, the number of Streptococcus mutans, lactobacillus and total colony-forming units (CFU) was estimated by the methods of Westergren and Krasse (1978). Dark field microscopy is used to analyze the supra- and subgingival plaque pathogenicity (the ratio of motile to non-motile bacteria in subgingival plaque samples is approximately 1:50; whereas in periodontally diseased sites, the ratio is approximately 1:1 (Listgarten and Hellden, 1978). Plaque accumulation and

gingival status were scored as previously described (Tinanoff et al., 1978); enamel white spot lesions and DMFS (decayed, missing, filled surfaces) were scored on each subject as well as photographed.

From the initial data collection, the patients were systematically divided into two groups, a NaF mouthrinse group and a SnF_2 mouthrinse group with regard to the number of bacteria in the saliva, i.e., every patient in the control group is balanced with a patient in the experimental group were asked to rinse with 10 ml of SnF_2 (200 ppm F^-) mouthrinse twice a day*. Patients in the control groups use 10 ml of NaF (200 ppm F^-) mouthrinse twice a day**. Patients who didn't wish to cooperate with daily rinsing but were willing to participate in the study constituted a second control group. The study is performed double-blind except for those subjects in the second control group***.

3. After one, three, and six months, new saliva and plaque samples were collected which were analyzed. Gingival and plaque indices were also performed. Between day 40 and 70, all patients saw the dental hygienist for a series of 3 visits--2 for oral hygiene instructions and mechanical prophylaxis and 1 for oral hygiene re-instruction (Table 1). All patients during the study period are being provided with complete restorative care by a dental resident at the patient's expense. The only variations from routine treatment that their assigned dentists are told is that the research hygienist will perform all preventive procedures as well as oral hygiene instruction. The above procedures are performed to keep the population as uniform as possible with regard to preventive procedures.
4. After 12 months, the same evaluation done at 1, 3, and 6 months was carried out. Furthermore, examination and photographing of previously noted enamel white spots will be performed.

5. Examinations at 18 months will consist of saliva and plaque samples as well as gingival plaque indices.

6. Evaluations at 24 months will be the same as those at 12 months.

At every examination point, the patients' mouths are inspected for any abnormality (oral lesions) and this data is recorded at each visit.

To monitor compliance with the mouthrinse regimens, each patient brings in the unused portion of the mouthrinse for "refill". The remaining mouthrinse is measured to determine compliance with the 20 ml/day usage. If a patient is not using the rinse as directed, he/she will either terminate from the study or be placed in the second control group.

Thus far we have started 34 patients in the study and 6 of these have dropped out (Table 2). Because no patient is delayed more than 30 days from initial visit until starting fluoride mouthrinsing, we have utilized multiple start-ups. Therefore, we have now had 12 patients completing the 6-months exam; 18, the 3-month exam; 29, the 1-month exam; and 34, the initial exam (Figure 2).

---

*Johnson and Johnson Dental Products Division produced for us at no charge 0.4% SnF_2 in glycerine base. Because of the instability of this agent as an aqueous solution, the patients mix 2 ml of the concentrate with 8 ml of water to produce the 200 ppm F^- rinse (pH 2.9). Each patient is given 150 ml of the concentrate each month.

**Daves Rose Hoyte has supplied us at 1/4 cost ($500) with Phos Flur Oral Rinse (200 ppm F^-, pH 4.0). Each patient is supplied with 1,000 ml of this rinse each month.

***Because of "human use" constraints, we could not deny any subject use of the fluoride mouthrinse. Hence, the necessity for the three group design.

TABLE 1: FLOW CHART OF CLINICAL MOUTHRINSE STUDY

NAME ______

\# ______

Screening ______

______ Initial approach to study
______ Patient interest
✓ Contact with study DH
✓ Explain study, consent form signed
10-31-80 Saliva sample
done Full mouth radiographs
______ Provider assignment
11-7-80 Appoint for initial scoring

00 Exam 11-7-80

✓ Saliva sample
✓ Plaque sample
✓ Plaque index
✓ Gingival index
✓ Plaque removal
✓ Clinical caries charting
✓ Clinical photos
2 Bottles Fluoride distribution
12-04-80 Appoint for 01 scoring

Phone ______

______ Check rinse compliance

Phone ______

______ Confirm 01 appointment

01 Exam 12-04-80

✓ Saliva sample
✓ Plaque sample
✓ Plaque index
✓ Gingival index
good Fluoride compliance
2 Refills
______ Appoint for D.H. in 10 days

D.H. 1 ______

✓ Explain gingivitis, periodontitis and rampant caries and their relation to plaque and sugar.
______ Diet History form given
______ Plaque disclosed, 60 %
✓ TBI (Bass, Modified Bass, Scrub)
✓ Maxilla (Scale, Rt. Plane)
excellent Fluoride compliance

D.H. 2 ______

good Review patient information
excellent Review diet history
______ Plaque disclose, 10 %
______ Review TBI, Interproximal cleaning
good (2) Check fluoride compliance
✓ Mandible (Scale, Rt. Plane)

D.H. 3 ______

excellent Review OHI
✓ Check root planning, polish
excellent Check fluoride compliance
______ Appoint for 03 exam

03 Exam 2/12/81

✓ Saliva sample
✓ Plaque sample
✓ Plaque index
✓ Gingival index
Good Fluoride compliance
2 Dispense bottles

Phone ______

______ Dispense bottles, mail

Phone ______

______ Dispense bottles, mail

Phone ______

______ Dispense bottles, mail
5-28-81 Appointment for 06 exam

06 Exam 5-28-81

✓ Saliva sample
✓ Plaque sample
✓ Plaque index
✓ Gingival index
✓ Clinical photos
______ Fluoride compliance
______ Appoint for 6 months recall

DH 6MR	12MR	18MR	24MR	
✓				Review Med & Dent. Hx.
✓				Oral exam & charting
good				OHI review, changes
not ind.				PBW Radiographs
✓				Scale, rt. plane, pol[illegible]
✓				Caries check by D.[illegible]
✓				Refer for restor[illegible]
✓				Fluoride compliance
✓				Dispense bottles

Phone (months)

______	7	______	14
______	8	______	16
______	9	______	18
______	10	______	20
______	11	______	22
______	12	______	24

12 Exam ______	24 Exam ______
______ Saliva sample	______ Saliva sample
______ Plaque sample	______ Plaque sample
______ Plaque index	______ Plaque index
______ Gingival index	______ Gingival index
______ Pocket depth	______ Pocket depth
______ Caries chart	______ Caries chart
______ Clinical photo	______ Clinical photos
______ Fl. compliance	______ Fl. compliance
______ Dispense bot.	______ Dispense bot.
______ Appoint for recall	______ Appoint for recall

TABLE 2: Patients, Visits, Time, Progress of Clinical Mouthrinse Study

			#Subjects				
00 Scoring Date	Series	Initial	01	03	06	12	24
11-07-80	100	14	12	9	9		
11-20-80	200	4	4	4	3		
1-13-81	300	5	5	5			
2-26-81	400	4	4				
3-26-81	500	4	4				
5-28-81	600	3					
Patients Initial Total		34					
Patients Drop Out		6					
Active Patients Total		28					

Date: 5-29-81

27 Active Patients

166 Visits

45 Minutes = length of average visit range from 30-90 minutes.

125.5 hours in patient contact

RESULTS

Since the study is in progress no data synthesis has taken place. The following 7 pages constitutes data from one subject as an example of the collection process: (The photographic series taken at the beginning of the study and at 6 months are not included.

NAME Spearrow, Gary PAT.# 112

1980	1981
Date 11-7-80 Age 25 Sal. Flow 1ml p.min	Date ____ Age ____ Sal. Flow ____
Number S. mutans: ____	Number S. mutans: ____
Lactobacilli: ____	Lactobacilli: ____
White spots: ____	White spots: ____
Surfaces at risk: ____	Surfaces at risk: ____
DMFS: ____	DMFS: ____
CFU ____	CFU ____

Reversals
Increased lesions
new lesions

Teeth	Primary	X-ray	Fillings	Teeth	Primary	X-ray	Fillings
M S	M O D B L	M D	M O D B L	M S	M O D B L	M D	M O D B L
18				18			
17	1 A			17			
16	1	2		16			
15	1			15			
14	1			14			
13	1			13			
12	1			12			
11				11			
21				21			
22				22			
23				23			
24				24			
25		1		25			
26	1			26			
27	1 14			27			
28				28			
38	1			38			
37	29			37			
36	A A 15			36			
35				35			
34				34			
33				33			
32				32			
31				31			
41				41			
42				42			
43				43			
44				44			
45	81			45			
46	A A 12			46			
47	15			47			
48				48			

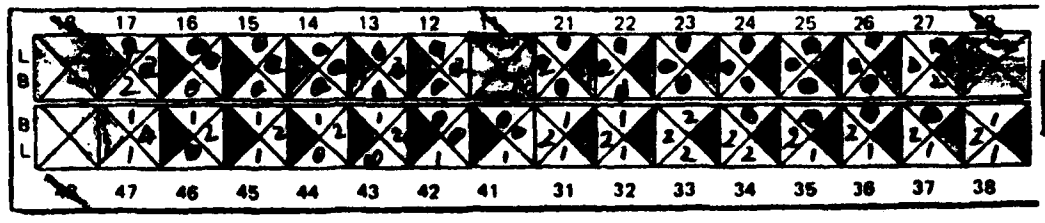
Sal. Samp. 12:10
Pl. Samp. 1:00
Score
Pad
Photo
FL.

MOUTHRINSE STUDY

NAME GARY SPEARROW # 112

VISIT # 02 DATE 11-07-80

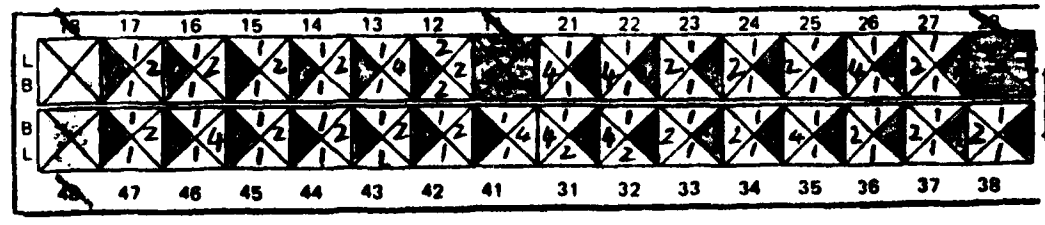
P. I.



$\bar{X}$ .82 ± .58

Freq.	
0	26
1	75
2	7
3	

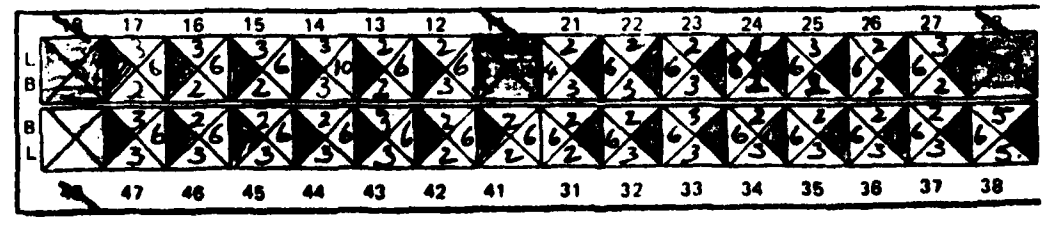
G. I.



$\bar{X}$ 1.16 ± .39

Freq.	
0	0
1	90
2	18
3	

POCKET DEPTH



$\bar{X}$ 2.84 ± .64

Freq.	
0	0
1	
2	28
3	75
4	3
5	4
6	

Comments:

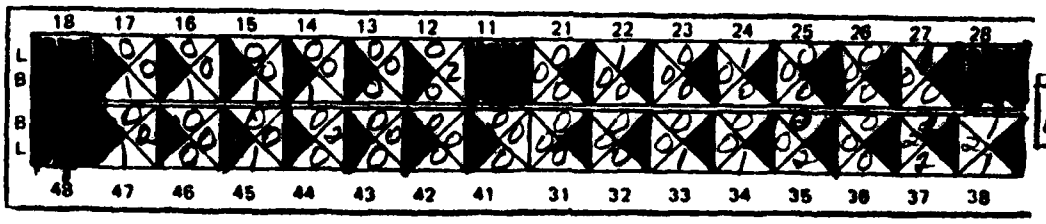
Saliva ✓
Plaque
Fluoride ✓

MOUTHRINSE STUDY

NAME Spearow, Gary # 112

VISIT # 01 DATE 12-04-80

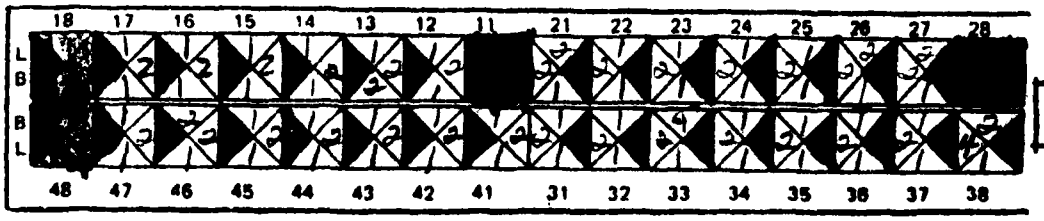
P.I.



$\bar{X}$ 0.25 ± .47

Freq.	
0	86
1	24
2	2
3	0

G.I.



$\bar{X}$ 1.01 ± .25

Freq.	
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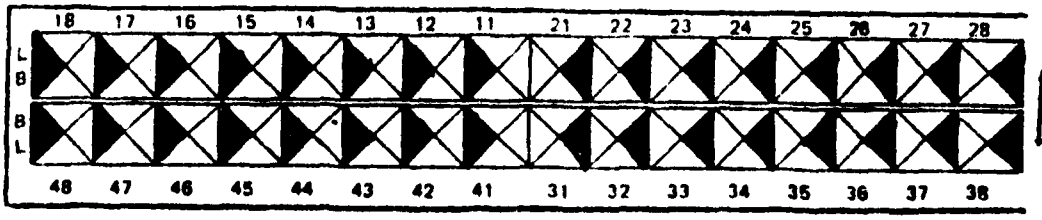
1	104
2	8
3	0

POCKET DEPTH



$\bar{X}$

Freq.	
0	
1	
2	
3	
4	
5	
6	

Comments:

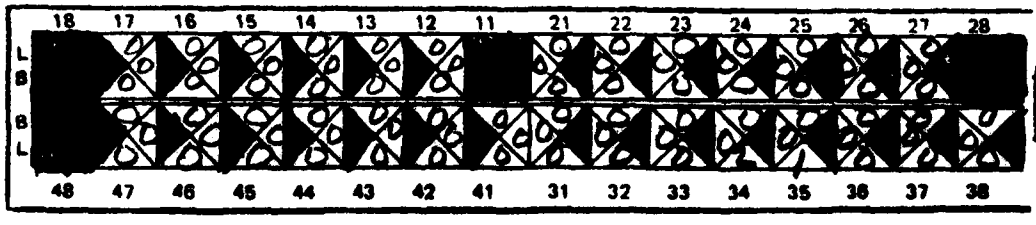
Saliva ✓
Plaque ✓
Fluoride Disp: 2 Ret: 0
Total 1 month

MOUTHRINSE STUDY

NAME Spearow, Gary # 112

VISIT # 03 DATE 2/12/81

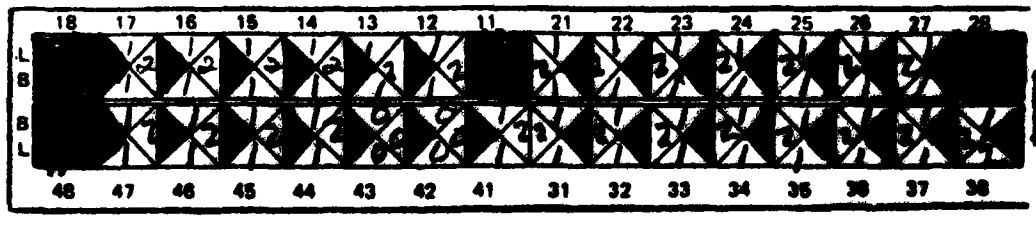
P.
I.



$\bar{X}$ .026 ± .21

Freq.	
0	110
1	1
2	1
3	

G.
I.



$\bar{X}$ .99 ± .8

Freq.	
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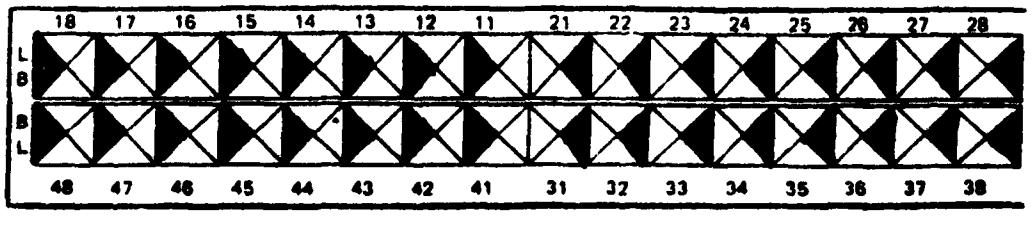
1	100
2	2
3	

POCKET DEPTH



$\bar{X}$

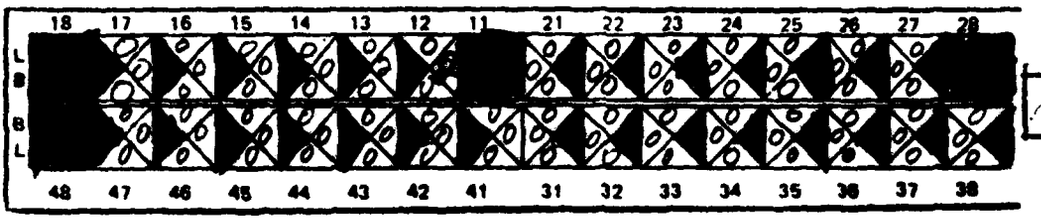
Freq.	
0	
1	
2	
3	
4	
5	
6	

Comments:

MOUTHRINSE STUDY

NAME Spearow, Gary # 112

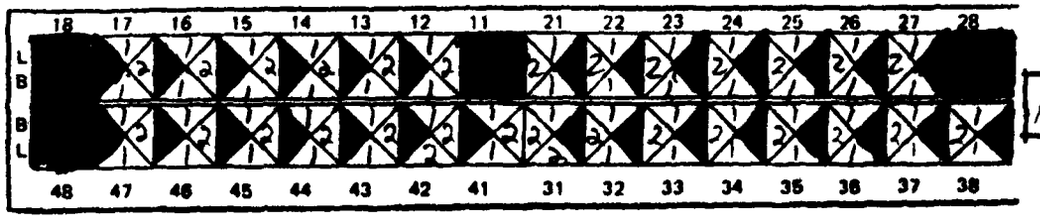
VISIT # 06 DATE 5/28/81



$\bar{X}$ 1.18

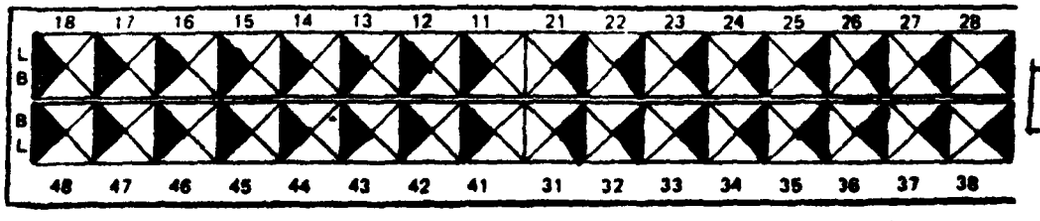
Freq.	
0	108
1	4
2	
3	

112



$\bar{X}$ 1.01

Freq.	
0	
1	110
2	2
3	



$\bar{X}$

Freq.	
0	
1	
2	
3	
4	
5	
6	

Comments:

NAME Spearow, Gary PAT. # 112

MICROBIOLOGICAL DATA

Scoring	Date	Total Aerobic	S. mutans	Lactobacilli
00	11-7-80	28.0	1.20	0
01	12-4-80	11.3	0.04	0.016
03	2/12/81	5.6	0.48	0.016
06	5/28/81	9.6	0.132	0?
09				
12				
15				
18				
21				
24				

Patient # 113

DARK FIELD DATA

Date _____

		Month 0	Month 1		Month 3		Month 6	Month 12
Supragingival (A) #36	1.				44		28	
	2.				30		47	
	3.				27		20	
	4.				0		5	
	5.				2		0	
	6.				0		0	
Subgingival (B) #36	1.				42			
	2.				30			
	3.				18		no sample	
	4.				3			
	5.				3			
	6.				4			
Supragingival (C) #46	1.	54	47	7↓	58	4↑	29	
	2.	24	14	10↑	22	2↓	43	
	3.	6	30	24↑	15	9↑	23	
	4.	6	4	2↓	2	4↓	5	
	5.	4	3	1↓	2	2↓	0	
	6.	8	9	1↑	1	7↓	0	
Subgingival (D) #46	1.	24	51	26↑			20	
	2.	14	12	2↓			22	
	3.	2	22	20↑			34	
	4.	4	5	1↑			17	
	5.	36	4	32↓			1	
	6.	20	6	14↓			6	

1. Coccoid
2. Str. rods
3. Filaments
4. Fusiform
5. Spirochetes
6. Motile rods

Antiplaque Properties of Sustained Release SnF_2

Introduction

The effective delivery of antimicrobials as well as other chemotherapeutic agents for the prevention or treatment of bacterial infections of tooth surfaces may be suboptimal due to its reliance on patient cooperation. Conventional methods for delivering of agents to the oral cavity involve use of mouthrinses, gels, and dentifrices (Ainamo, 1977), and these systems are compromised in varying degrees due to their reliance on patient cooperation for repeated applications of the chemotherapeutic agent (Mirth and Bowen, 1976).

Interest in sustained release systems for drug delivery in medicine and dentistry has been increasing. Besides taking the repeated administration of a drug away from patient responsibilities, controlling the rate and site of release may be a more effective means of administering a drug. To date, sustained release systems in dentistry have involved delivering: steroids for the management of apthous ulcers (Yeoman, Greenspan, and Harding, 1978); anti-fungal drugs for the management of denture stomatitis (Douglas and Walker, 1973; Thomas and Nutt, 1978), antibacterials for the control of plaque (Addy, 1981) and fluorides for the control of dental caries (Mirth and Bowen, 1976; Daperon and Jodrychowski, 1980; Forsten, 1976; Zitz, Gedalia, and Grajower, 1981; Whitford et al., 1980; Friedman, 1980; Mirth et al., 1981). To date, the largest clinical study has been performed with an Trilaminate methacrylate sodium fluoride-releasing device which is attached to the buccal surfaces of the teeth. The intraoral device was found to elevate the levels of fluoride in plaque, saliva and urine, but had no effect on plaque or gingival parameters (Mirth et al., 1981).

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism (Hamilton, 1977) as well as reacting physicochemically with enamel to reduce enamel soluability or remineralize initial caries (for review, see Mellberg, 1976). Yet only stannous fluoride has been shown to reduce the quality of plaque at concentrations compatible with frequent oral use (for review, see Tinanoff and Weeks, 1979). Based on its demonstrated ability to inhibit plaque, stannous fluoride was chosen as the active agent whose effectiveness might be enhanced by incorporation into a sustained release delivery system.

The purpose of these pilot studies was to: (1) develop an SnF_2 intraoral sustained release delivery system; (2) assess the release of active agent and mechanical properties in vitro; and (3) evaluate the delivery system in vivo for antiplaque properties, oral fluoride release, and systemic effects.

Methods and Materials

In Vitro Tests

Cement Preparation

To four dental cements--zinc phosphate cement (Improved powder, type I., S.S. White), polycarboxylate cement (Durelon, Premier), Intermediate Restorative Material (Caulk) and zinc-oxide and eugenol (generic)--stannous fluoride (Ozark-Mahoning) was added (W/W powder) to produce ratios of 20, 40, and 60 percent. Because of pilot studies, stannous fluoride was also added to polycarboxylate powder at a 70 percent ratio. Prior to incorporating the SnF_2 into the cement, the fluoride crystals were pulverized to a fine powder by triturating the crystals in an amalgamator (Silomet) for 1 minute at maximum velocity.

The cements with or without addition of the SnF_2 powder were mixed by one operator as recommended by the manufacturer; i.e., zinc phosphate was mixed on a glass slab using incremental additions of powder to liquid over a 2 minute period; polycarboxylate was mixed on a coated paper pad (Durelon) and spatulated for 30 seconds; IRM was mixed on an absorbant paper pad incrementally and thoroughly spatulated; zinc oxide eugenol was mixed with the same technique as IRM.

Compressive Strength of Cements

After the appropriate mixing of the cement formulations, each sample was used to fill three 10 x 20 mm plastic capsules (Beem Capsules, size 00, Polysciences, Inc.). Following several days to allow for complete set, the cements were removed from the capsule and the ends ground parallel on a sandpaper wheel to a standard height of 7.3 mm. Ultimate compressive strengths of the samples were measured on a materials testing instrument (Instron, Model 1113, Canton, MA) with a crosshead speed of 0.5 cm/min. Some selected specimens which underwent a 30 day fluoride leaching trial

were also tested for post-leaching compressive strength.

Leaching of Fluoride from Cements

A cylindrical specimen of each fluoride concentration from the four cements was prepared, removed from the mold, and then coated with blue inlay wax (Kerr Products, Emeryville, CO) so that only the open, circular end was exposed. (The poor set and low compressive strength of IRM allowed only testing of 20% SnF_2 in this cement).

Each sample was separately incubated at 37° in 250 ml normal saline. After 24 hr., the saline was discarded saving only 2 ml of the solution for fluoride analysis. Each flask containing the specimens was again refilled, incubated, and this process was repeated for 30 days to enable characterization of the leaching of fluoride from each cement. After the 30 day period, the 310 fluoride samples collected were prepared for measurement by diluting them 1/1 with ionic strength buffer (TISAB with CDTA; Orion Res., Cambridge MA). The fluoride concentrations were then determined using a fluoride electrode (Orion 90-09 A) connected to a digital readout electrometer (Orion 70) comparing the samples to NaF standards.

In Vivo Tests

Subject

Since 70% SnF_2 in polycarboxylate cement demonstrated favorable leaching properties while maintaining compressive strength leaching properties (see results), *in vivo* pilot studies on one subject (N.T.) were performed to assess the antiplaque properties of the released fluoride from this cement. After human consent approval, an "MOD" amalgam was removed from a lower right 2nd molar and an orthodontic band was cemented and the tooth restored with the 70% SnF_2-polycarboxylate cement.

Two days prior to placement of the temporary restoration, the subject obtained complete plaque removal by means of a toothbrush with the aid of

disclosing solution. The subject then abstained from all forms of active oral hygiene for the next 2 days. On day 0 of the experiment (2 days of no oral hygiene), the teeth were stained with disclosing solution (Trace, Lorvic Corp., St. Louis, MO) and photographs (1:2) of the buccal tooth surfaces were taken. After the temporary restoration was placed, the teeth again were made plaque free and another 2-day no oral hygiene period was begun, terminated by photographs of the plaque and then complete plaque removal. This sequence of 2-day no oral hygiene period and photographs of plaque formation was continued for the 1 month experimental period and post-experimental period of 3 successive months. At the end of the 1 month experimental period, the temporary containing SnF_2 was removed and replaced with polycarboxylate cement without SnF_2.

Plaque Scores

The 4 slides taken on each of the 26 experimental periods were used to determine the extent of visual deposits on the teeth. Plaque scoring was performed according to the method described by Martens & Meslin (1972) using only the buccal surfaces of 20 teeth (from 2nd premolars to 2nd premolar of both maxillary and mandibular arches). The intra-oral slides were examined using a 7x magnifier and a radiographic viewbox. After calibration of 2 examiners (N.T. & T.S.), scoring was performed independently and the mean of the 2 scores was obtained. Both "total deposits" as well as "globular deposits" were recorded. Globular deposits were defined as those deposits that appeared to have thickness and texture. Scores were reduced to mean score per tooth, and a mean score of 5 represents deposits on all surfaces.

Salivary and Urinary Fluoride Levels

To determine salivary and urinary fluoride levels, whole saliva and urine samples were obtained prior to and each day of the 1 month experimental period. Whole saliva samples and urine samples were collected at

the same time of each day prior to, during and following the experimental month. Samples were frozen to prevent bacterial growth and warmed to room temperature before fluoride measurements.

SEM and Percent Stannous Fluoride Remaining in Temporary

A fragment of the removed SnF_2-polycarboxylate temporary that was removed after 1 month was prepared for scanning electron microscopy. After coating the specimen with gold-palladium, it was examined with a Hitachi H300 with a H3010 scanning attachment at 20 KV. Following microscopy, the sample was weighed, pulverized and suspended in equal parts of deionized water (50 cc's) and TISAB II with CDTA (50 cc) for 24 hours. The solution was then assayed for fluoride ion concentration and the percent of stannous fluoride remaining after one month was calculated.

Results

In Vitro

Compressive Strength

The control samples of polycarboxylate, zinc phosphate, IRM and zinc oxide eugenol, i.e., those without addition of SnF_2, showed compressive strengths of 23.0±1.3, 14.1±2.7, 5.3±1.5, and 0.7 Klb_5/in^2, respectively. The compressive strengths of the cements were decreased linearly with addition of SnF_2 to the powder component of the cement. Yet, polycarboxylate cement still maintained relatively high compressive strength even with large additions of fluoride (Figure 1). Zinc phosphate cement appeared to be more detrimentally affected by the SnF_2. IRM and zinc oxide eugenol had initial low compressive strengths and the addition of SnF_2 inhibited the setting reaction to the extent that these materials were made unsuitable for further preparation.

In the "post-leaching" compressive strength test, SnF_2-polycarboxylate cement, again, was least affected. For example, 60% SnF_2 in polycarboxylate versus 60% SnF_2 in zinc phosphate cement produced post-leaching compressive strengths of 6.2 vs 0.4 Klb_5/in^2, respectively. While the unleached 70% SnF_2 in polycarboxylate cement was found to have 10.5±1.5, the post-leached 70% SnF_2 in polycarboxylate cement samples had a compressive strength of 5.9 Klb_5/in^2.

Release of Fluoride from Cement

Release of fluoride from the SnF_2-cement mixtures showed that 70% SnF_2 in polycarboxylate cement had the best release over 30 days with a meal of 3.7±2.8 ppm F^-/day. The release of fluoride from both polycarboxylate and zinc phosphate cements was rather consistent each day. In all cases, greater release was found in polycarboxylate cement when compared to zinc phosphate (Figure 2). The mean fluoride release from the IRM and zinc oxide eugenol

cements was unimpressive, ranging from 0.1 to 0.4 ppm F/day.

In Vivo

Anti-Plaque Effects

The baseline scoring, i.e., no oral hygiene for 2 days without SnF_2 temporary in place, showed a "total" plaque score of 3.5±0.08 and a globular plaque score of 2.28±0.06. During the experimental month, the total plaque was 2.9±0.43 and the globular plaque was 0.96±0.25 (Figure 3). In the month following the experimental period, total plaque returned to baseline levels; whereas, globular plaque displayed a small "carry over" effect (Table 1). The only side effects noted were a slight metallic taste on the first day and brown staining on the dorsum of the tongue adjacent to the temporary. No staining of the teeth was evident.

Salivary and Urinary Fluoride Levels

The pre-experimental baseline for salivary and urinary fluoride were 0.039±0.015 and 1.6±0.5, respectively. The mean salivary fluoride level, during the experimental month, was increased to 1.86±1.32 ppm F and the urinary fluoride levels returned to normal daily fluctuation after the first week; whereas, salivary fluoride levels were noted to be most elevated throughout the first half of the month (Figure 4). The relationship between salivary fluoride levels and globular plaque scores is shown in Figure 5. There was not a linear relationship between the elevation in salivary fluoride level and the reduction in the globular plaque score as evidenced by the weak correlation coefficient r+-.3.

SEM and Percent F in Removed Temporary Restoration

Scanning electron micrographs of the temporary restoration, removed after one month, showed small spaces in the cement in the areas approximate to the orthodontic band. The surface of the restoration, exposed to the

oral environment, had an amorphous surface with no visual holes (Figure 6). Fluoride analysis of the 20 mg sample revealed that 8.3 mg SnF_2 remained or 41.5% of the restoration's weight was assumed to be SnF_2.

Integrity of the Restoration

The marginal adaptation and wear of the 70% SnF-polycarboxylate was not substantial during the experimental period. The temporary had color change from pale pink to speckled black to ultimately a uniform grey (Figure 7).

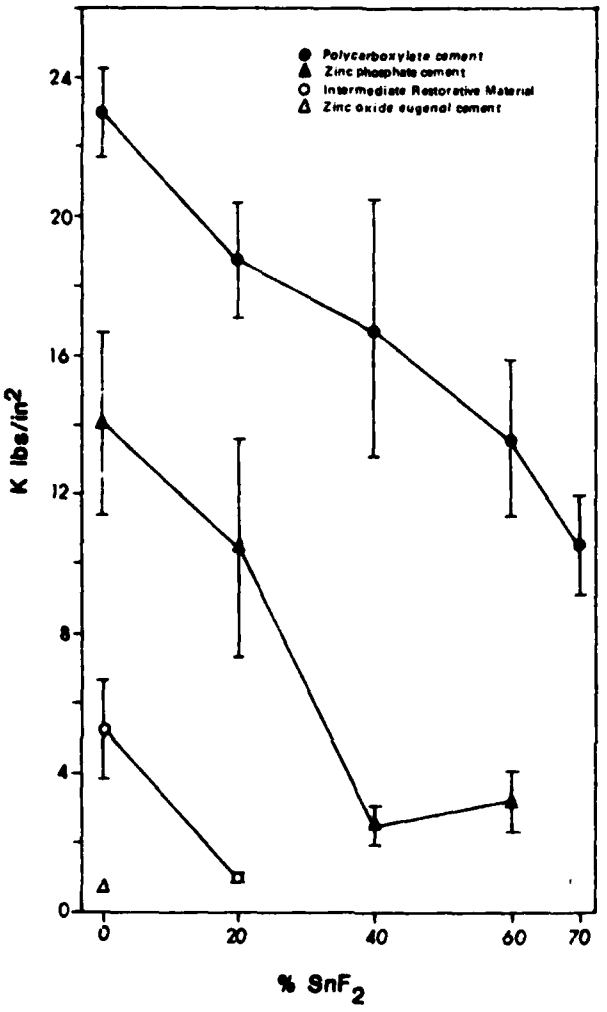


Figure 1: Ultimate compressive strength (mean ± S.D.) of 4 dental cements containing from 0 to 70% SnF_2.

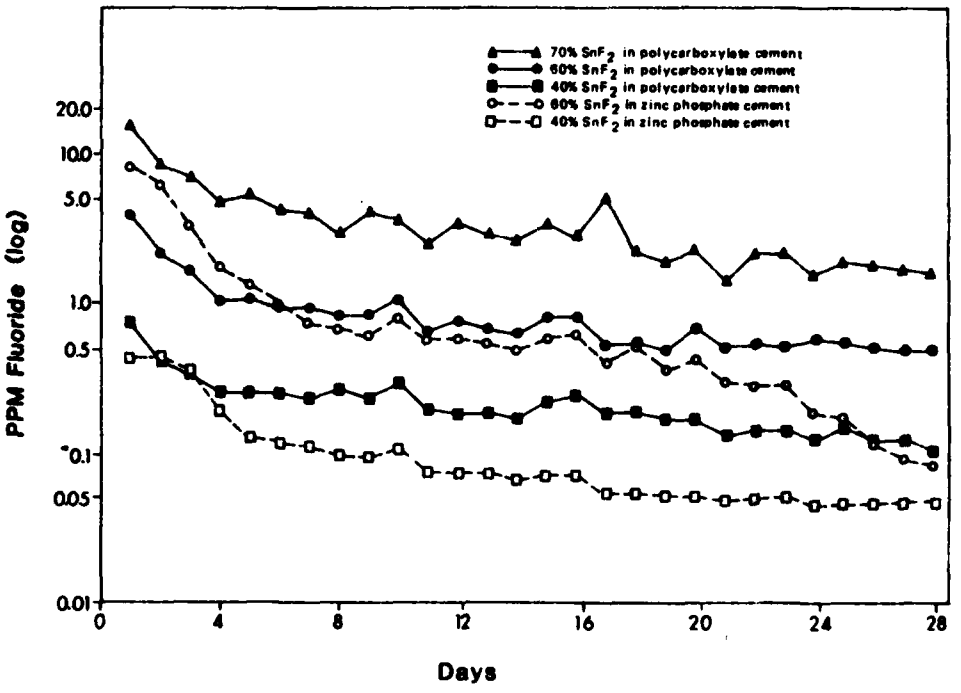


Figure 2: In vitro release of fluoride from 2 dental cements containing 40 to 70% SnF_2.

	Total Plaque	Globular Plaque
Baseline Period	3.5 ± .08	2.28 ± .58
Experimental Period	2.9 ± .43	0.96 ± .25
Month Following Experimental Period	3.5 ± .13	2.08 ± .29

Table 3: Total and glubular plaque scores (mean ± S.D.) prior to, during, and after the 30 experimental period.

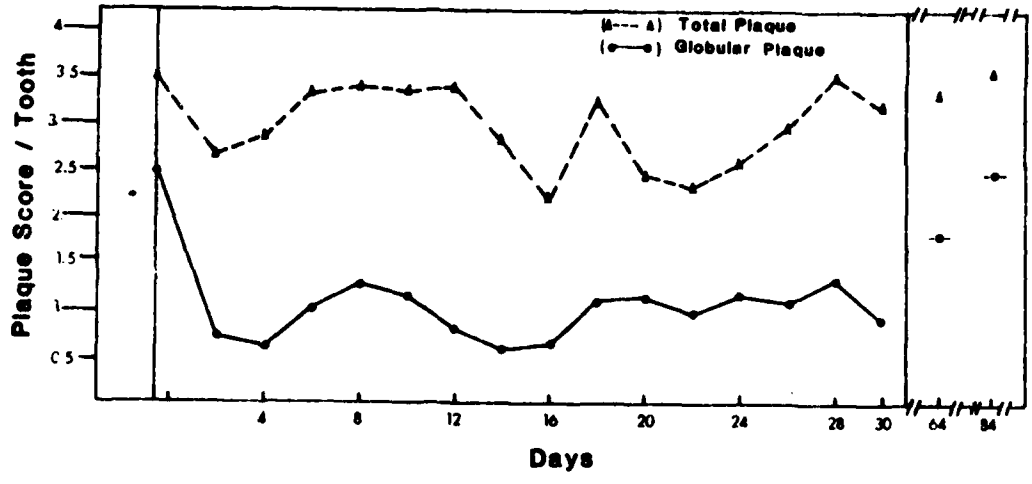


Figure 3: Visual plaque (total and globular) scores from subject during the 30 day period with the sustained release fluoride restoration in place and approximately 1 and 2 months after the restorations had been removed.

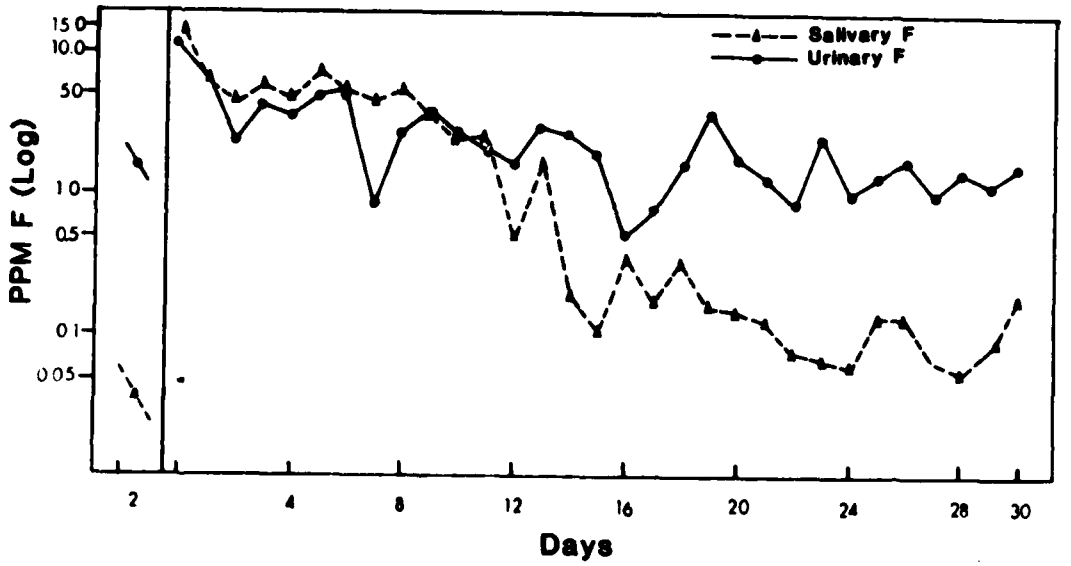


Figure 4: Daily fluoride concentration in saliva and urine from subject, in the 2 day baseline period and in the 30 day period with the sustained release fluoride restoration in place.

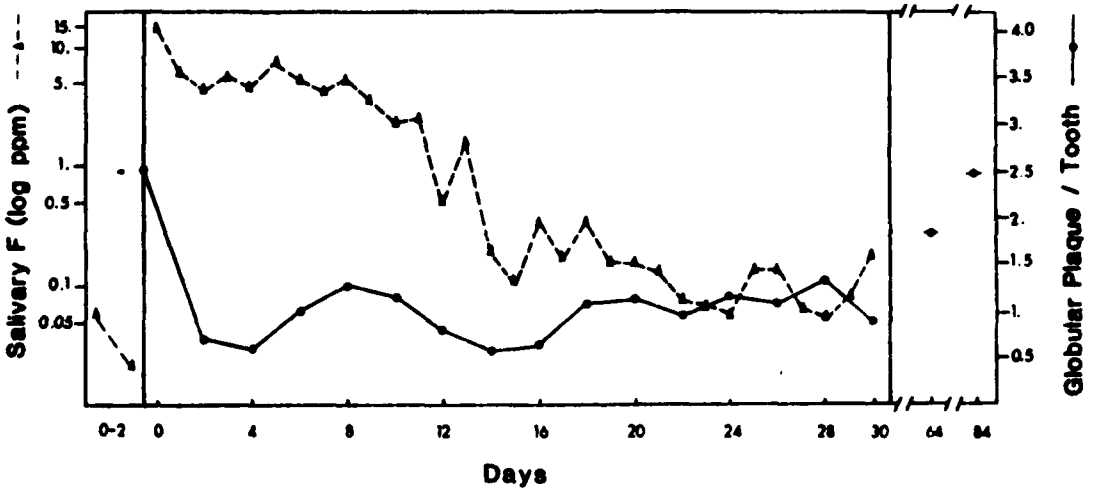


Figure 5: Relationship between salivary fluoride levels and globular plaque scores in one subject during the 30 day test period.

Figure 6: Condition of the sustained release temporary restoration at the end of the 30 day experimental period.

DISCUSSION

These preliminary sustained release experiments designed to evaluate an intracoronal (within the tooth) SnF_2 delivery system both *in vitro* and *in vivo* showed that besides liberating fluoride for the one month test period, the released fluoride had measurable antiplaque properties in the one test subject.

As shown by the ultimate compressive strength tests, the compatibility of large additions of pulverized SnF_2 in polycarboxylate cement was remarkable. Others have reported that additives such as alumina and SnF_2 can actually increase strength of polycarboxylate cement (Smith, 1978). Even though we found 70% SnF_2 in polycarboxylate cement reduced the compressive strength by about one-half, clinically, the material showed sufficient strength in the one month test period. In our mechanical tests, we did not follow exact ADA specifications for testing dental cements (ADA spec. #8) and consequently, our results vary from others.
However, the different testing procedures would not affect the relative results of one cement tested with various concentrations of fluoride.

The *in vitro* tests to examine the release pattern of fluoride from the various cement demonstrated that fluoride leached from these materials in a consistent pattern. The release of fluoride was elevated in the first few days for all cements and the release levels were related to the percent SnF_2 in the cements. Due to the favorable release patterns and compressive strength of polycarboxylate cement with 70% SnF_2, we obtained human use approval for *in vivo* trials in one subject using this cement as a intercoronal restoration.

The 30 day, one subject trial of the 70% SnF_2-polycarboxylate temporary restorations showed an initial peak release of fluoride followed by a longer sustained release comparable to that which was similar to the *in vitro* trial.

In the first day, the fluoride content of the saliva reached 15 ppm F and the level gradually declined over the one month. The lowest recorded fluoride level in saliva, 0.1 ppm F on day 28, was still substantially higher than the 0.05 ppm F baseline. The mean salivary fluoride level for the month of 1.86 ppm F was similar to the 30 day mean of 1.45 ppm F reported by Mirth et al., 1981, from their trial with the trilaminate fluoride-release device cemented to the buccal surface of maxillary molars.

Even though the fluoride levels in saliva were markedly high initially and remained elevated during the experimental month, the urinary fluoride levels were only noteable for the first 2 days. By comparing the weight and fluoride content of the initially placed restoration to that removed after 30 days, the total fluoride injestion was estimated to be no more than 57 mg fluoride or 1.9 mg per day. (The actual amount was less due to loss of cement with mixing and occlusal adjustment). The brief elevation of urinary fluoride and systemic fluoride injestion was found to be inconsequential in one subject. Yet since nephrotoxic levels of inorganic fluoride in urine, peak or duration, have not been established, it may be prudent at this time to continue fluoride release trials in subjects without renal disease (Taves et al., 1970).

In contrast to other studies (Mirth et al., 1981), antiplaque properties were noted in this 30 day trial, probably as a result of using SnF_2 instead of NaF. Besides the antiplaque properties at mouthrinse concentrations (Tinanoff et al., 1980), SnF_2 at levels compatible with slow intraoral release, 10 ppm F, have been shown to reduce the number of S. mutans that can adhere to wires yet increase this organism's production of extracellular polysaccharides (Ferretti, Tanzer, and Tinanoff, 1981). The increase in extracellular polysaccharide formation and the clinical observation of increased pellicle-like deposits in those subjects rinsing with

SnF_2 (Tinanoff and Weeks, 1980) made us discriminate between total plaque and globular plaque (essentially bacterial deposits). (We have previously noted by phase contrast microscopy that deposits on teeth that appear flat and textureless have few bacteria among amorphous matrix.) The marked reduction in globular plaque in the experimental period suggests that there may be fewer bacteria present in the deposits on the tooth surfaces due to the presence of SnF_2. Further clinical trials using bacteria per miligram plaque parameter are necessary to confirm the finding of less bacteria on teeth of subjects exposed to sustained release SnF_2.

Clinically, the SnF_2-polycarboxylate restoration had no unfavorable properties in the 1 month trial. Aside from the staining of the tongue, no local or systemic side effects were noted. Moreover, the integrity and wear of the restoration was not significant. The intercoronal site of release allowed for good retention while not being bulky. The disadvantage of the location is that a patient must have a carious lesion or defective restoration in a tooth that can be used for the site prior to placement of a permanent restoration. However, this drawback would probably not limit its applicability in patients needing this type of therapy. Based on the favorable release of fluoride, mechanical properties, and putative anti-plaque properties of the SnF_2-polycarboxylate temporary restoration, clinical trials using microbiologic as well as clinical parameters are indicated to assess the feasibility of this system as an adjunct in the control of caries and periodontal disease.

Antiplaque Determinants of SnF_2: pH and Ions

INTRODUCTION

The pathology of dental caries and periodontal disease is associated with the accumulation of bacterial plaque on teeth (Löe, 1969). It is well established that bacterial metabolism can be altered by fluoride ions, even at 1.0 part/10^6 (Hamilton, 1977), yet only SnF_2 has been shown to reduce the quantity of plaque at concentrations compatible with frequent oral use. The more potent antiplaque effect of SnF_2 could be related to: its low pH which may alter bacterial membrane permeability by inhibiting enzyme reactions (Eisenberg and Marquis, 1980); the hydrogen-fluoride (HF) produced at low pH which may be more effectively transported across the membrane (Whitford, et. al., 1977); or the divalent cation, Sn++, which may interfere with bacterial adhesion and/or cohesion (Skjorland, Gjeomo and Rölla, 1978).

Past studies in our laboratory suggest that the bacterial accumulation of the heavy metal tin, coupled with the fluoride effect, may be responsible for the antiplaque properties of SnF_2 (Tinanoff and Camosci, 1980). The purpose of these experiments was to examine the effect of several divalent cations either as fluoride or chloride salts, and to test them at various pH's inorder to segregate the antiplaque parameters of SnF_2. The variables used to determine the various antiplaque properties of intermittent exposures of several cations either at low or high pH's and either as fluoride or chloride salts were: (1) bacterial acid production, (2) visual plaque scores, (3) dry weight of the bacteria, (4) amount of cation/mg of dry plaque weight and (5) cellular location of cations using electron microscopy and microprobe analysis.

MATERIALS AND METHODS

Stock cultures of a streptomycin-resistant mutant of Streptococcus mutans NCTC 10449, Bratthall serotype c, were maintained by monthly transfer in fluid thioglycollate medium (Difceo) supplemented with meat extract (20% v/v) and excess $CaCO_3$. This strain is known to be cariogenic in rats (Tanzer, 1979) and to produce heavy plaque in vitro (Tinanoff, Tanzer and Freedman, 1978). Moreover, serotype c is representative of the most frequently found human serotype in Europe and in the U.S.A. (Bratthall, 1972; Shklair and Keene, 1973). For the experiments, cultures were adapted to growth in the complex medium of Jordan, Fitzgerald and Bowler (1960), (pH 7.5) supplemented with 50 mg of Na_2CO_3/l and containing 5% sucrose.

The following fluoride solutions at a concentration of 250 parts/10^6 F^- were tested at various pH's to determine their ability to alter the colonization of bacteria on wires: sodium fluoride (0.055% NaF); stannous fluoride (0.103% SnF_2); stannic fluoride (0.064% SnF_4); zinc fluoride (0.068% ZnF_2); and 100 parts/10^6 F^- lead fluoride (0.065% PbF_2). PbF_2 was tested at 100 parts/10^6 F^- concentration due to incomplete aqueous solubility of 250 parts/10^6 F^-. The fluoride solutions were also tested against equimolar cation controls as chloride salts to examine the metal ion effect. Table 1 summarizes the pH, cation and anion concentrations used for each agent.

Culture tubes containing 10ml of Jordan's medium supplemented with 5% sucrose were inoculated with 0.1ml of an adapted culture of S. mutans NCTC 10449. Stainless steel wires (0.03"), inert to bacterial growth (Gristina, 1976), were used as a substratum for plaque growth (Tinanoff and Camosci, 1980). The wires, suspended by rubber stoppers, were placed in each inoculated tube and agitated to evenly distribute the organisms. After 12 hours incubation at 37°, the wires were removed from the medium, and exposed for 1 minute to the appropriate pH adjusted solution.

The exposure was followed by a 1 minute non-agitated rinse in 10 ml deionized H_2O (pH 6.0) before transfered to fresh medium. The water rinse was performed to reduce carryover of the test solution into the fresh growth medium. Exposures to the solutions were repeated every 12 hrs. to simulate a twice daily mouth-rinsing regimen (Fig. 7). Twelve hours after the last exposure of the plaques to their respective agent, a pH measurement of the growth medium was used to determine the acid production by the bacteria. Also 12 hrs. after the last exposure to the test solutions, the thickness of the bacterial plaques on the wires were visually scored by the method of McCabe, et.al., 1967. The plaque from each wire was then collected in pre-weighed tubes, pelleted by centrifugation and excess water removed. After the samples were dried for 3 days at 70° , the tubes were reweighed to calculate dry plaque weights. Atomic absorption spectrophotometry was used to determine the metal content of the dry plaque samples. For tin analysis, the samples were suspended in known quantities of 10% HCl and compared to tin standards ($SnCl_4$, Alfa Div., Danvers, MA). The samples and standards were measured in triplicate using an atomic absorption spectrophotometer (Perkin-Elmer, Model 403) equipped with a graphite furnace (HGA-74) (Trachman, Tyberg and Branigan, 1977). Plaque samples measured for the presence of lead were performed in similar nature to that of tin (Christian and Feldman, 1970). Zinc was quantified by the method of addition using flame atomic absorption spectrophotometry (Manning, 1975). A deuterium lamp was used in all cases to correct for non-atomic absorption signals.

Transmission electrons microscopy was utilized to observe the bacterial morphology of the plaques exposed intermittently to H_2O, NaF, SnF_2, SnF_4, $SnCl_2$, PbF_2, $PbCl_2$, ZnF_2 and $ZnCl_2$. After 48 hrs., the specimens were removed from the growth medium, fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4, 390 mOsm) and postfixed in 1% osium tetroxide in Veronal buffer (pH 7.3) (Warshawsky and Moore, 1967). The specimens were then washed in the phosphate buffer. After serial dyhydration in acetone and embedment in epoxy medium (Spur, 1969), the

resin was polymerized at 70°C. Thin sections were prepared with LKB ultra-microtonic using a diamond knife. Silver-gold colored sections were examined unstained with a Seiss EM/10 electron microscope at 80 kv.

Those unstained specimens which showed electron dense material were further examined by a transmission electron microprobe. These analyses were performed in JEOL JEM-100 CX transmission electron microscope equipped with a high resolution electron microscope accessory (ASID) and a Kevex Si(Li) X-ray dectector connected to a Micro-X Analytical X-ray Spectrophotometer, Model 7000. The spectrophotometer was linked to a Texas Instrument Data Terminal Printer.

Areas for analyses were located and photographed in the transmission mode (TEM), and then in the scanning mode (STEM) which was operated at an accelerating voltage of 80 keV. From the Polaroid photographs of the STEM image, the location of the probe spot was recorded. For microprobe analysis, the specimens were tilted with an eucentric goniometer of 30° for optimum collection of X-rays. The electron beam was focused to a small spot (6-10 nm) and positioned on the areas to be analyzed. X-rays emanating from these areas were counted for 100 s. The X-ray energy spectrum was displayed on the spectrophotometer and also recorded on Polaroid film.

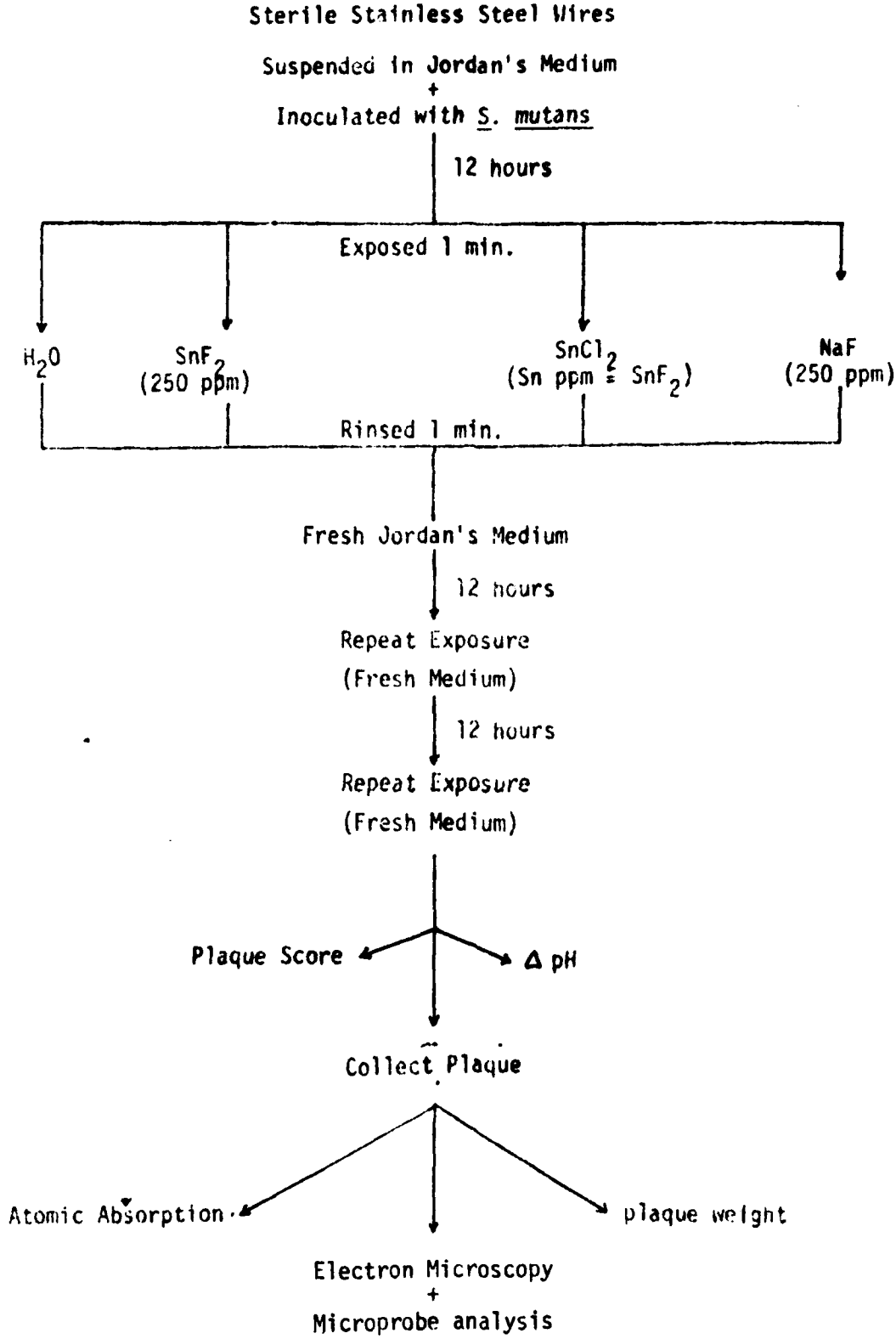


Figure 7: Flow diagram used to test the effect of various agents (1 min./ 12 hrs. for 48 hrs.) on S. mutans (Table). Same design was used to test other agents listed in Table .

Agent	Cation (ppm)	Anion (ppm)	pH
NaF	303	250	2.0, 2.5, 3.0, 5.5, 6.0, 7.0.
$SnCl_2$	783	463	2.5, 7.0.
SnF_2	783	250	2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0.
SnF_4	390	250	2.3, 5.0.
ZnF_2	428	250	5.2.
$ZnCl_2$	428	465	4.9.
PbF_2	545	100	3.0, 6.0.
$PbCl_2$	545	187	3.0, 6.0.
H_2O	---	---	2.5, 7.0.

Table 4: List of the solutions at various pH's which were exposed (1 min/12 hrs.for 48 hrs.) to wire adherent S. mutans NCTC 10449. Fluoride solutions were tested at 250 parts/10^6 F^-, except for PbF_2 which was tested at 100 parts/10^6 F^-. The cations in the comparable chloride salts were equimolar to the fluoride salts.

RESULTS

Acid Production

In our initial studies we found that wire adherent S. mutans exposed for 1 minute, twice daily, for two days to SnF_2 showed inhibition of plaque growth and acid production (Δ pH of growth medium), whereas NaF or $SnCl_2$ exposures showed little effect (Table 5).

Subsequently, more precise experiments were performed to test whether the effectiveness of SnF_2 was due to pH, cations, or a combination of factors. In these trials, intermittent exposures of plaques to SnF_2 at pH's below 4.0 virtually inhibited plaque formation and thereby lowering the amounts of bacterial acid produced (Tables 6 and 8). This inhibitory effect decreased as the pH of SnF_2 was increased with SnF_2 adjusted to pH 5.0 or 6.0 not effective in inhibiting acid production. The reduced bacterial acid production found in SnF_2 tested plaques directly correlated ($r = 0.94$) to the reduction of plaque weight on the wires (Fig. 8).

Although SnF_2 was most effective at a lower pH, even at pH 5&6 its effectiveness in reducing pH drop was greater than the agents tested (Tables 7 & 8). The fluoride salts of lead and zinc slightly reduced acid production by comparison to their chloride salts; however, the Δ pH of those plaques exposed to the fluoride salts of lead and zinc were equal to NaF at neutrality. NaF at a lower pH was found to be slightly more effective than NaF near neutrality in inhibiting acid productions (Tables 5, 6, and 7,). In contrast to SnF_2, SnF_4 had little or no effect on acid production.

Plaque Scores

The one minute, twice daily exposures of S. mutans to all the test agents for two days, including SnF_2, pH 5.0 and 6.0, had little effect on the organism's capacity to attach and proliferate on the stainless steel wires. However, the bacteria exposed to SnF_2, pH 2.0, 3.0 and 4.0 consistantly showed less plaque formation as measured by visual plaque scores (Table 6). Figure 2 compares the

differences in plaque growth of NaF and SnF_2 at a low pH.

Plaque Weights

Except for SnF_2 at pH's 2.0 through 4.0, the other test agents showed no effect in reducing the amount of dry plaque weights in comparison to NaF (Table 5 , 6,and 7). Bacterial plaques exposed to SnF_2 solutions equal to or less than pH 4.0, averaged two-thirds less plaque weight than the low pH exposed plaques of NaF (Table6).

Metal/mg Plaque

Initial studies of intermittent exposures of SnF_2 and $SnCl_2$ on S. mutans at neutral and acidic pH's suggested a pronounced effect with SnF_2 at low pH's. Associated with the plaque reduction produced by low pH SnF_2 solutions was the large uptake of tin within the plaques. A greater uptake of tin was found when the plaques were exposed to $SnCl_2$ at a low pH, however, antiplaque properties were not evident (Table 5).

The experiment designed to observe the effect of varying the pH of SnF_2 and NaF on bacterial acid production, plaque formation and tin uptake found an inverse relationship between plaque weight and tin quantity in the plaques exposed to SnF2 pH's 3.0 through 6.0 (R = -0.91) (Table 6; Fig. 8).

Plaques exposed to lead salts showed small accumulations of the metal in the samples (Table 7) while those plaques exposed to zinc salts had only trace amounts (Table 8). Although a strong correlation exists between bacterial tin uptake versus the decreasing pH of SnF_2 solutions, no difference in metal uptake was found by altering the pH of the lead and zinc salts (Tables 7 and 8).

In contrast to the large tin uptake in bacteria exposed to SnF_2, SnF_4 exposed plaques contained much less tin, and varying the pH of the SnF_4 did not have an effect on the tin uptake (Table 8).

Agent	pH	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Sn/mg Plaque (μg)
H_2O	2.5	2.80	4	11.0 ± 0.2	N.D.[β]
	7.0	2.73	4	10.7 ± 0.5	N.D.
NaF	2.5	2.54	4	12.5 ± 0.5	N.D.
	7.0	2.72	4	11.2 ± 0.9	N.D.
$SnCl_2$	2.5	2.74	4	12.3 ± 0.6	17.5 ± 6.8
	7.0	2.75	4	11.1 ± 0.7	3.3 ± 0.5
SnF_2	2.5	1.69	2	7.2 ± 2.0	107.0 ± 37.5
	7.0	2.58	4	13.0 ± 0.5	6.2 ± 0.7

α Scored by McCabe method

β None detected

N = 3; $\bar{x}$ ± S.D.

Table 5: Initial study of intermittent exposures (1 min/12 hrs for 48 hrs.) of tin and/or fluoride solutions on acid production, plaque formation, and tin accumulation of wire adherent S. mutans NCTC 10449. Deionized water (pH 2.5 and 7.0) was used as a control. Fluoride solutions at 250 ppm F^-; cations in $SnCl_2$ equal to SnF_2.

Agent	pH	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Sn/mg Plaque (μg)
NaF	3.0	1.80	4	6.4 ± 0.9	N.D.[β]
	6.0	2.41	4	6.5 ± 0.3	N.D.
SnF_2	2.0	0.18	<1	1.8 ± 0.1	λ
	3.0	0.24	<1	2.4 ± 0.5	42.9 ± 7.1
	4.0	0.47	1	2.6 ± 0.5	36.9 ± 3.6
	5.0	0.99	3	5.7 ± 0.4	20.1 ± 0.5
	6.0	1.63	4	5.9 ± 0.8	3.6 ± 0.7

[α] Scored by McCabe method

β None detected

λ Laboratory accident

N = 3; $\bar{x} \pm$ S.D.

Table 6: Effect of intermittent exposures (1 min/12 hrs for 48 hrs.) of NaF (pH 2.0 and 6.0) and SnF_2 (pH 2.0 to 6.0) on acid production, plaque formation, and tin accumulation of wire adherent S. mutans NCTC 10449. Fluoride solutions at 250 ppm F^-.

Agent	pH	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Pb/mg Plaque (μg)
NaF	3.0	1.94	3	5.70 ± 0.2	N.D.[β]
	6.0	2.33	3	4.60 ± 1.0	N.D.
$PbCl_2$	3.0	2.04	3	6.10 ± 0.3	2.2 ± 0.2
	6.0	2.26	3	5.50 ± 0.9	1.2 ± 0.2
PbF_2	3.0	1.90	3	6.73 ± 0.3	3.5 ± 0.5
	6.0	1.99	3	5.50 ± 0.1	2.4 ± 0.7

[α] Scored by McCabe method

[β] None detected

N = 3; $\bar{x}$ ± S.D.

Table 7: Effect of intermittent exposures of PbF_2 and $PbCl_2$ compared to NaF on acid production, plaque formation, and lead accumulation of wire adherent S. mutans NCTC 10449. Test solutions adjusted to either pH 3.0 or 6.0. Fluoride solutions at 100 ppm F^-; cations in $PbCl_2$ equal to PbF_2.

	Agent pH	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Sn/mg Plaque (μg)
NaF	5.5	2.74	3	9.8 ± 0.6	N.D.[β]
SnF_2	3.5	0.27	<1	1.3 ± 0.4	39.1 ± 1.4
SnF_4	2.3	2.56	3	10.9 ± 0.2	8.9 ± 2.0
SnF_4	5.0	2.56	3	10.4 ± 0.3	6.1 ± 0.3
ZnF_2	5.2	2.75	3	10.0 ± 0.5	0.05 ± 0.01
$ZnCl_2$	4.9	2.87	3	9.0 ± 0.2	0.12 ± 0.07

α Scored by McCabe method

β None detected

N = 3; $\bar{x} \pm$ S.D.

Table 8: Effect of intermittent exposures (1 min/12 hrs for 48 hrs.) of NaF, SnF_2, SnF_4, ZnF_2 and $ZnCl_2$ on acid production, plaque formation, and metal accumulation of wire adherent S. mutans NCTC 10449. Natural pH for all test solutions, except SnF_4 which was adjusted to pH 5.0. Fluoride solutions at 250 ppm F^-; cations in $ZnCl_2$ equal to ZnF_2.

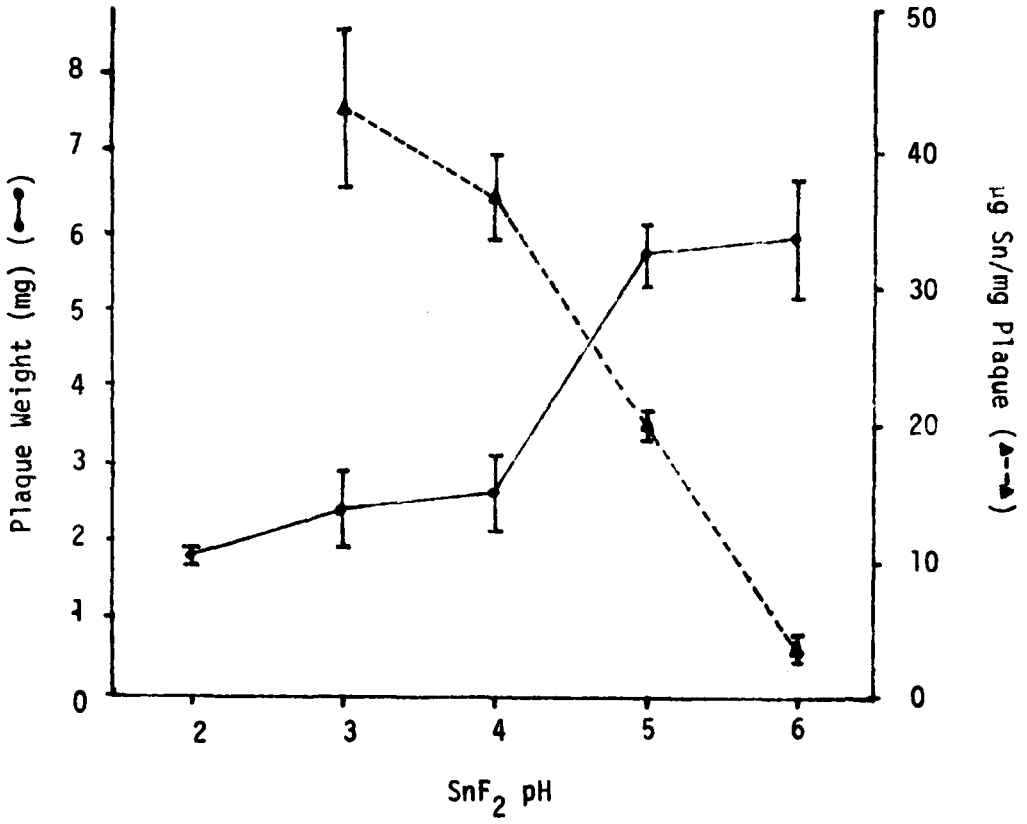


Figure 8: Intermittent exposure of SnF_2 (1 min./12 hrs. for 48 hrs.) at pH 2.0-6.0 on growth (plaque weight) and metal uptake (Sn/mg plaque). Reduction of bacterial growth by SnF_2 is inversely related to metal present in the bacteria at varying pH's.

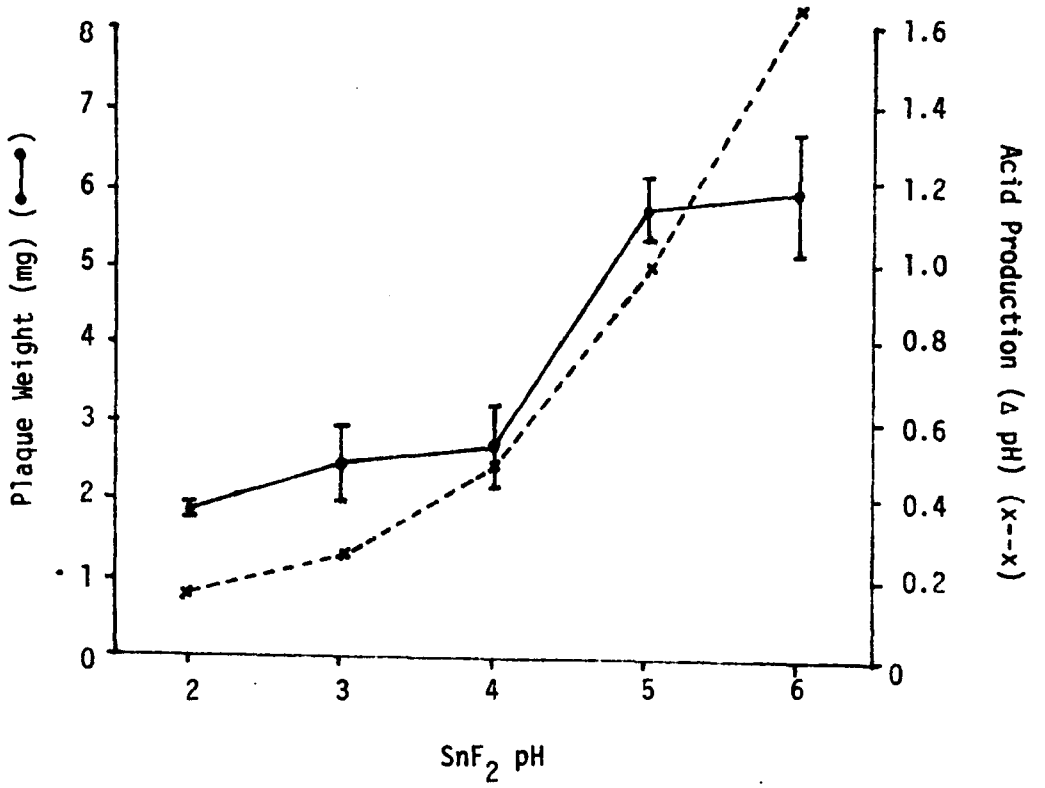


Figure 9: Intermittent exposures of SnF_2 (1 min./12 hrs. for 48 hrs.) at pH 2.0-6.0 on growth (plaque weight) and acid production (ΔpH) of S. mutans NCTC 10449. SnF_2 was most effective $\leq$ pH 4.0. Acid produced by bacteria correlated to the amount of plaque growth on wires.

Appendix A

List of Publications and Presentations
Supported by U.S. Army Contract DAMD 17-78-C-8066

Papers Published:

1. Tinanoff, N., Hock, J., Camosci, D., and Hellden, L. The Effect of Stannous Fluoride Mouthrinse on Dental Plaque. J. Clinical Periodontology 7:232-241, 1980.

2. Tinanoff, N. and Weeks, D. Current Status of SnF_2 as an Antiplaque Agent. Pediatric Dentistry 1:199-204, 1979.

3. Hock, J. and Tinanoff, N. Resolution of Gingivitis in Dogs Following Topical Applications of 0.4% Stannous Fluoride and Toothbrushing. J. Dent. Res. 56:1652-1653, 1979.

4. Tinanoff, N. and Camosci, D. Microbiological, Ultrastructural and Spectroscopic Analyses of the Anti-Tooth-Plaque Properties of Fluoride Compounds In Vitro. Arch. Oral Biol. 25:531-543, 1980.

Papers Submitted:

*1. Ferretti, G. A., Tanzer, J. M. and Tinanoff, N. The Effect of Fluoride and Tin on S. Mutans Viability, Growth, Acid and Glucan Production. Caries Research

*2. Ferretti, G. A. and Tinanoff, N. Ultrastructure of S. Mutans Grown in Media Supplemented with Fluoride and Tin. Caries Research

3. Fisher, J. G., Tanzer, J. M., Tinanoff, N. and Paulakis, V. Plaque formation by LTA-deficient oral streptococci. Infect. Immun.

Papers in Preparation:

1. Camosci, D. A. and Tinanoff, N. Antiplaque Determinants of SnF_2: pH and Ions. Antimicrob. Agents Chemother.

2. Swanson, T. D. and Tinanoff, N. Antiplaque Properties of Sustained Release SnF_2. Pediatric Dentistry

Presentations:

1. Tinanoff, N., Camosci, D. A. and Gross, A. Intermittent Exposure of Fluorides on Bacterial Colonization of Enamel In Vitro. Proc. 57th Ann. Meet. Int. Assoc. Dent. Res., New Orleans, 1979.

2. Tinanoff, N. and Tanzer, J. M. Electron Microscopy of Pellicle Formed by Enamel-Adherent Organisms. Proc. 57th Ann. Meet. Int. Assoc. Dent. Res., New Orleans, 1979.

3. Tinanoff, N., Hock, J., Camosci, D. and Hellden, L. Clinical Trial to Test Antiplaque Effect of SnF_2 Mouthrinse. Proc. 57th Ann. Meet. Int. Assoc. Dent. Res., New Orleans, 1979.

*Paper attached in appendix.

4. Camosci, D. A., Tinanoff, N. and Gross, A. Intermittent Exposure of Fluorides and Bacterial Colonization of Enamel In Vitro. Ann. Soc. Microbiol., Conn. Valey Branch, Storrs, CT, 1979.

5. Tinanoff, N. and Camosci, D. A. Intracellular Electron-Dense Granules Associated with SnF_2 Treated Plaque. Proc. 58th Ann. Meet. Am. Assoc. Dent. Res., Los Angeles, 1980.

6. Camosci, D. A. and Tinanoff, N. Quantity of in in Dental Plaque Exposed to SnF_2 and $SnCl_2$. Proc. 58th Ann. Meet. Am. Assoc. Dent. Res., Los Angeles, 1980.

7. Ferretti, G. A., and Tinanoff, N. Bacteriostatic and Bactericidal Effects of Different Fluoride Compounds on S. Mutans. Proc. 58th Ann. Meet. Amer. Assoc. Dent. Res., Los Angeles, 1980.

8. Young Pavlakis, V., Tanzer, J. M. and Tinanoff, N. Plaque formation by LTA-deficient Oral Streptococci. Proc. 58th Ann. Meet. Amer. Assoc. Dent. Res., Los Angeles, 1980.

9. Tinanoff, N. Anitplaque properties of SnF_2. The Album Society, Philadelphia, PA., April 18, 1980.

10. Tinanoff, N. Influence of Metal Ions on Microbial Structure. European Research Group of Oral Biology, Merseyside, England, April 25-27, 1980.

11. Tinanoff, N. Stannous Fluoride as an Antiplaque Agent. The University of Liverpool, Dental School, England, April 25, 1980.

12. Tinanoff, N. The Antiplaque Properties of SnF_2 Mouthrinse. The University of Bern Dental School, Switzerland, April 30, 1980.

13. Camosci, D. A. Quality of Tin in Dental Plaque Exposed to SnF_2 and $SnCl_2$. Am. Soc. Microbiol., Connecticut Valley Branch, Bridgeport, Connecticut, 1980.

14. Tinanoff, N. Antiplaque Properties of SnF_2 Mouthrinse. Research Section. American Academy of Pedodontics. May 26, 1981.

15. Ferretti, G. A. The Effect of Low Dose Fluoride on Bacterial Metabolism, Growth, and Attachment. Thesis Award. American Academy of Pedodontics. May 25, 1981

16. Camosci, D. A. and Tinanoff, N. Effect of SnF_2 and NaF at Variable pH on In Vitro Plaque Formation. Connecticut Chapter of the Amer. Assoc. of Dent. Res. March 9, 1981.

17. Camosci, D. A. and Tinanoff, N. Effect of SnF_2 and NaF Variable pH on In Vitro Plaque Formation. Connecticut Valley Branch of Amer. Soc. Microbiol. April 10, 1981.

18. Tinanoff, N. and Camosci, D. A. Antiplaque Properties and Bacterial Uptake of PbF_2 and $PbCl_2$. Proc. 59th Ann. Meet. Int. Assoc. Dent.Res., Chicago, 1981.

19. Swanson, T. D., Goldberg, A. J. and Tinanoff, N. Antiplaque Properties of Controlled Release SnF_2. Proc. 59th Ann. Meet. Int. Assoc. Dent. Res., Chicago, 1981.

20. Ferretti, G. A. and Tinanoff, N. Ultrastructural Changes of S. Mutans Exposed to Fluoride and Tin. Proc. 59th Ann. Meet. Int. Assoc. Dent. Res., Chicago, 1981.

Appendix B

Published Abstracts of Papers Presented in 1981.

ABSTRACT FORM
INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
and
AMERICAN ASSOCIATION FOR DENTAL RESEARCH

59th General Session and Annual Session—Chicago, Illinois, March 19-22, 1981

ABSTRACTS MUST BE RECEIVED BY OCTOBER 6, 1980.
ABSTRACTS RECEIVED AFTER OCTOBER 6, 1980, WILL BE RETURNED TO SENDER.

Complete: Items 1-4 below and type abstract within box, following instructions on reverse side.

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1. Full name and address of **PRESENTER.**

T. D. Swanson
Department of Pediatric Dentistry
School of Dental Medicine
UCONN Health Center
Farmington, CT 06032

IADR member? yes ☐ no ☒

2. Mode of presentation:

- ☒ oral presentation only
- ☐ poster presentation only
- ☐ oral or poster mode acceptable

3. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?

☐ yes ☐ no ☒ read by title acceptable

4. **Group Classification and Key Word Descriptors.**
(Transfer choices from Descriptor Form. Use letter/number codes ONLY. Minimum, five; maximum, 10.)

Letter	Number
A	10
B	10
C	5
F	4
H	8
J	1
K	5
N	6

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Antiplaque Properties of Controlled Release SnF_2. T.D. SWANSON*, A.J. GOLDBERG, and N. TINANOFF, UCONN School of Dental Medicine Farmington, CT

SnF_2, used twice a day as a mouthrinse, has been shown to have significant antiplaque properties. The purpose of this study was to: 1) develop a temporary restorative material which could slowly release SnF_2; and 2) test this material in vivo for antiplaque properties.
Polycarboxylate cement was found through compressive strength testing to be least affected by the addition of large percentages of SnF_2. For example, equal quantities of SnF_2 (wt SnF_2/wt powder) in polycarboxylate and zinc phosphate cement produced a compressive strength of 13.6 vs. 3.2 lbs/in^2, respectively. The SnF_2 incorporated into polycarboxylate cement was also found through in vitro slow release studies to liberate over a 1 month period 3.7 ±2.8 ppm F^-/day.
The polycarboxylate-SnF_2 cement was then used in a molar "M.O.D." temporary restoration of one subject (NT). Salivary and urinary fluids were collected daily; plaque scores (2 examiners) were performed for a month at 2 day intervals of no oral hygiene. Baseline salivary F^- levels of 0.04 ppm were increased to 1.9 ±2.3 ppm over the month period. From a plaque baseline score of 2.5 (out of a total possible score of 5), globular plaque decreased in the experimental month to 0.95 ±0.25. Loss of integrity of the restoration (marginal adaptation, wear) was not substantial during this period.
This controlled release temporary, besides giving elevated salivary fluoride levels over the 30 day experimental period, produced marked reduction in visual plaque.

Supported by U.S. Army Contract DAMD 17-78-C-8066

51

5. Reviewer's Ratings:	6. Disposition:
☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐ ☐
1 2 3 4 5	O P T R W

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ABSTRACT FORM
INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
and
AMERICAN ASSOCIATION FOR DENTAL RESEARCH

59th General Session and Annual Session—Chicago, Illinois, March 19-22, 1981

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Submit: Abstract form—Original and 6 Xerox copies. Complete Presenter card (blue) and one Co-author card (white) for each co-author on your abstract (one white Co-author card is provided).

1. Full name and address of **PRESENTER.**

IADR member? yes ☐ no ☐

2. Mode of presentation:

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- ☐ oral or poster mode acceptable

3. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?

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Letter	Number
A	

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Ultrastructural changes of S. mutans exposed fluoride and tin. G.A.FERRETTI*, U.of Kentucky, Lexington, KY and N.TINANOFF, UCONN School of Dental Med., Farmington, CT

Previous studies noted antiplaque effects of various fluoride compounds (>100ppm F) on bacterial plaque ultrastructure. The purpose of this investigation was to observe and to quantitate the ultrastructural findings associated with growth of S. mutans in medium supplemented with low concentrations of various fluoride- or tin-containing compounds.
Stainless steel wires suspended in growth media containing 10 ppm F as NaF, SnF_2, Na_2SnF_6 or TiF_4 were inoculated with 0.1 ml of an adapted culture of S. mutans NCTC 10449S. $SnCl_2$ equimolar to Sn in SnF_2 served as a control for tin; the same volume of H_2O as the other solutions served as another control. After a three day incubation period, (37°C, with transfer to fresh media every 24 h), the wires with attached microorganisms were processed for electron microscopic observation and semiquantitative analysis.
Increase in extracellular material was observed in all specimens cultured with fluoride. Electron lucent holes (polyphosphate), a sign of unbalanced growth were found in 32% of those bacteria in the $SnCl_2$ group and in 81% of the bacteria in the SnF_2 group. Electron dense granules (tin) were found intracellularly in 10% of the bacteria in the $SnCl_2$ test group and in 23% of the SnF_2 test group. The intracellular tin accumulation and polyphosphate found in those bacteria exposed to SnF_2 may be distinguishing ultrastructural features of the antiplaque properties of stannous fluoride.
This study was supported by U.S. Army Contract DAMD 17-78-C-8066.

5. Reviewer's Ratings:	6. Disposition:
☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐ ☐
1 2 3 4 5	O P T R W

52

(over)

ABSTRACT FORM
INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
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59th General Session and Annual Session—Chicago, Illinois, March 19-22, 1981

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Submit: Abstract form—Original and 6 Xerox copies. Complete Presenter card (blue) and one Co-author card (white) for each co-author on your abstract (one white Co-author card is provided).

1. Full name and address of **PRESENTER.**

Norman Tinanoff
Dept. of Pediatric Dentistry
School of Dental Medicine
UCONN Health Center
Farmington, CT 06032

IADR member? yes [X] no [ ]

2. Mode of presentation:

- [X] oral presentation only
- [ ] poster presentation only
- [ ] oral or poster mode acceptable

3. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?

[ ] yes [ ] no [X] read by title acceptable

4. **Group Classification and Key Word Descriptors.**
(Transfer choices from Descriptor Form. Use letter/number codes ONLY. Minimum, five; maximum, 10.)

Letter	Number
A	10
B	19
C	2
G	7
H	8

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Antiplaque Properties and Bacterial Uptake of PbF_2 and $PbCl_2$. N. TINANOFF* and D.A. CAMOSCI, University of Connecticut School of Dental Medicine, Farmington, CT

We have previously shown through electron microscopic observations large deposits of electron dense granules within bacteria exposed intermittently to SnF_2 (AADR Abst. 980, 1980); and atomic absorption quantitation has revealed 8x more Sn/mg plaque in plaques exposed to SnF_2 than $SnCl_2$, with greater antiplaque effect noted for SnF_2 (AADR Abst. 979, 1980). The purpose of these experiments was to treat plaque with PbF_2 and $PbCl_2$ to more fully understand the mechanisms of SnF_2.
Wires suspended in 10 ml of medium supplemented with 5% sucrose were inoculated with a S. mutans NCTC 10449 culture. Every 12 h the wires were removed from the medium, exposed for 1 min to either PbF_2 (100 ppm F^-), $PbCl_2$ (Pb equimolar to PbF_2) or NaF (100 ppm F^-) with all solutions adjusted to pH 3 and 6.
There were no differences in plaque scores, plaque weights or pH of the medium in any of the treatment groups. Using atomic absorption, PbF_2 (pH 3) had the highest μg Pb/mg plaque; however, plaques comparably treated with SnF_2 had approximately 4x more μg Sn/mg plaque. Electron microscopy of those groups exposed to Pb revealed lead deposits only associated with bacterial cell walls. No intracellular metal accumulation was noted with PbF_2 as was found with SnF_2. SnF_2 may be effective due to the heavy metal accumulation intracellularly.

Supported by U.S. Army Contract DAMD 17-78-C-8066

5. Reviewer's Ratings					6. Disposition				
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Appendix C

Copy of Papers Submitted to Caries Research.

To Koning
5/5/81

The Effect of Fluoride and Stannous Ions on S. Mutans:

I. Viability, Growth, Acid, and Glucan Production

G. A. Ferretti*
J. M. Tanzer
N. Tinanoff

Departments of Pediatric Dentistry and Oral Diagnosis
School of Dental Medicine
University of Connecticut Health Center
Farmington, Connecticut 06032

Adknowledgement: This study was supported by U.S. Army Contract DAMD 17-77-C-8066

Running Title: Effect of fluoride and stannous ions on S. mutans metabolism

Key Words: S. mutans, metabolism, antiplaque, SnF_2

Communication to: Norman Tinanoff, D.D.S., M.S.
Department of Pediatric Dentistry
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Department of Pediatric Dentistry
University of Kentucky
College of Dentistry
Lexington, KY 40506

ABSTRACT

The effects of various salts of fluoride and tin were assessed on S. mutans NCTC 10449S viability, growth, acid production, glucan, DNA formation, and tin accumulation. SnF_2 had more potent bacteriostatic and bactericidal effects than $SnCl_2$ or NaF, Na_2SnF_6 or TiF_4. SnF_2, $SnCl_2$, Na_2SnF_6 and NaF (at 10 ppm F or Cl) reduced the growth yield of S. mutans while acid production of this oganism appeared to be reduced only in the fluoride supplemented media. Bacteria grown in fluoride supplemented media had greater amounts of both the water soluble and alkali soluble glucans per bacterial mass, with SnF_2 having the greatest effect, increasing the water soluble component 10 times and the alkali soluble component 3 times over the controls. Greater tin uptake was noted in cells exposed to SnF_2 than those exposed to $SnCl_2$.

INTRODUCTION

In addition to its physicochemical interactions with tooth enamel, fluoride may influence plaque acid production, growth and attachment. Evidence of fluoride inhibition of acid production, even at 1 ppm, is well established (Bibby and Van Kesteren, 1940; Hamilton, 1974). Higher concentrations of fluoride may affect bacterial growth or viability, and clinical evidence suggests that daily topical application of 1.23% (12,300 ppm) fluoride as NaF (pH 3.0) reduces human plaque scores (Loesche, et al.. 1975). Stannous fluoride (100-1,000 ppm) applications reduce plaque in experimental animals (König, 1959; Hock and Tinanoff, 1979) and humans (Svatun, et al., 1977; Yankell, et al., 1980; Tinanoff et al., 1980). The more pronounced effect of SnF_2 than NaF on plaque formation may possibly be due to the former's effect on bacterial attachment (Tinanoff et al., 1976) and/or tin accumulation within bacterial cells (Tinanoff and Camosci, 1980).

Because most studies of fluoride or stannous ions have been performed at concentrations that could have been bactericidal or bacteriostatic, it appears valuable to examine their antiplaque properties at low levels (10 ppm) in order to differentiate between possible antiplaque mechanisms.

MATERIALS AND METHODS

Enamel Specimen Preparation

Enamel sections approximately 180 mm^2 were cut from smooth surfaces of bovine incisors using a diamond drill with water coolant. A hole was placed in each specimen so that a 0.030 inch diameter stainless steel wire could be used to suspend it in a culture tube. Specimens were cleaned with a slurry

of pumice to remove organic material, washed with deionized water in an ultrasonic cleaner, and autoclaved. Inlay casting wax (Kerr Products, Emeryville, CO) was used to cover the cut inner aspects of specimens leaving only the intact surface enamel exposed. The specimens were disinfected in 70% ethyl alcohol for 15 minutes and then rinsed in sterile deionized water for 10 minutes.

The surface area of the enamel slabs was estimated by making 1:1 photographic negatives of specimens and placing them over mm^2 blocked graph paper, the number of mm^2 blocks contained within the outline of the enamel specimen being approximately equivalent to the exposed enamel surface area of the specimen. This surface area exposed to test agents and bacteria was used for subsequent calculations.

Microorganisms and Agents

Streptococcus mutans NCTC 10449S (Tanzer et al., 1976) was selected as the test organism since this organism attaches to enamel in a similar way in vivo (Tinanoff et al., 1978); causes caries (Tanzer et al., 1976; Tanzer, 1979); and is representative of the most frequently found S. mutans serotype in human populations (Bratthall, 1972; Keene et al., 1977). Stock cultures were maintained by monthly transfer in fluid thioglycolate medium (Difco) supplemented with meat extract (20% v/v) and excess $CaCO_3$. For minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) determinations, cultures were adapted to and grown in trypticase soy broth (TSB; BBL). For all other experiments, stock cultures were adapted to and grown in complex medium (Jordan et al., 1960) supplemented with 5% sucrose and 50 mg/l Na_2CO_3. All experiments were performed at 37°C under microaerophyllic conditions.

Fresh aqueous solutions of several fluoride compounds were first prepared at 100 ppm with respect to F, i.e., NaF (0.022% w/v, pH 5.3), SnF_2

(0.041%, pH 3.8), Na_2SnF_6 (0.024%, pH 3.5), and TiF_4 (0.016%, pH 2.9), and then added to the complex medium supplemented to produce fluoride concentrations of 10 ppm. $SnCl_2$ (0.05%, pH 2.9), equimolar with respect to the Sn in SnF_2 (100 ppm F), was similarly prepared and added to the growth medium. As a F-free, Sn-free control, an equal volume of deionized water was added to the medium. The final pH of the supplemented media in all cases was 7.6.

To insure the accuracy of calculated nominal fluoride levels, free fluoride was determined by fluoride electrode (Orion 90-09A, Orion Research Laboratories, Cambridge, MA) immediately after addition of the fluoride agent to the media and after incubation of inoculated or uninoculated media for 24 hours at 37°C.

MIC/MLC Determination of Test Agents

To determine the concentration of the various fluoride or stannous solutions that could either kill or completely inhibit growth of S. mutans, the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of these agents (Barry, 1976) were determined. TSB culture tubes containing serial dilutions of the presumptive antimicrobials were inoculated such that there were 5.0×10^5 CFU/ml of strain 10449S. After incubation at 37°C for 16-18 hours, they were evaluated for tubidity. Because some test agents precipitated, uninoculated controls were used to establish baseline turbidity due to apparent chemical changes of the test agents in broth. The MIC was defined as the lowest concentration of an agent resulting in turbidity no greater than that of the corresponding uninoculated tube. The MLC was defined as the lowest concentration of agent resulting in failure to recover viable microorganisms from inoculated culture tubes at the end of 16-18 hours. Viability was tested by plating cultures on blood and Mitis Salivarius agars.

Bacterial Growth and Acid Production

To assess growth, the optical density of complex medium cultures containing the various agents at 10 ppm F (or 10 ppm Cl in the case of $SnCl_2$) was monitored at hourly intervals after inoculation by cultures adapted to the same medium without the test agents. Optical density was measured with a Spectronic 20 Spectrophotometer (Bausch and Lomb, Rochester, NY) at 600 nm. Simultaneously, the pH of the cultures was measured.

DNA/Glucan Analysis

NaF^-, SnF_2^-, $Na_2SnF_6^-$, and TiF_4^- supplemented media were placed into culture tubes containing the wire-suspended enamel cylinders and were inoculated with 0.1 ml of an S. mutans culture adapted to the same medium without the test agents. The enamel specimens were transferred serially every 24 hours to fresh media. After 3 days growth, the wax was removed from each enamel slab leaving bacteria attached only to the surface enamel. The enamel specimens were then sonified (Bronson Model W 185, Heat Systems Ultrasonics, Plainview, NY) with a microprobe tip in deionized water for 30 seconds at 50 watts with the output at 4, directing the probe tip such as to remove all bacterial aspects from the enamel surface, as judged microscopically. The dislodged bacteria were centrifuged (9,000 xg, 10 minutes, 0C) and resuspended in deionized water three times. A sample of the suspended cells and of the spent culture liquor of the third day's incubation was retained for glucan analysis according to the procedure of Freedman and Tanzer (1974). The remainder of the previously adherent cells and the spent culture fluid of the third day's incubation was analyzed for DNA after hot perchloric acid extraction (Ogur and Rosen, 1950; Burton, 1956; Tanzer, Wood, and Krichevsky, 1960).

Atomic Absorption Spectrophotometry

After 3 days growth, the bacteria on wires of each treatment group were

pooled into a pre-weighed glass centrifuge tube, pelleted by centrifugation, the the supernatant fluid removed. Samples were dried for 3 days at 70°C and the tubes re-weighed. After the dry weights of the harvested cells were thus calculated, the samples were suspended in 3.6 M. HCl. Tin in the samples and in standards ($SnCl_2$, Alfa Chemical, Danvers, MA) was measured in triplicate using a Model 403 atomic absorption spectrophotometer (Perkin-Elmer, Stamford, CT) equipped with an AGA-74 graphite furnace.

RESULTS

Fluoride Levels in Growth Media

The NaF- , Na_2SnF_6- , and SnF_2-supplemented media exhibited, by fluoride electrode, 10 ppm fluoride immediately after preparation, consistent with their nominal concentrations computed at the weighing of the compounds. After 24 hours incubation, however, all three showed a decrease of approximately 1 ppm F in both inoculated and uninoculated media, possibly due to organic binding of fluoride to constituents of the growth medium. Only TiF_4 did not have measured fluoride concentrations equal to their nominal levels; nominal 10 ppm solutions had measured levels of only 2.3 ppm F both in fresh medium and after 24 hours incubation of inoculated or uninoculated media.

MIC/MLC

SnF_2 had the lowest MIC and MLC of the fluoride compounds, 60 and 125 ppm F, respectively, when compared according to fluoride ion concentration (Table 1). TiF_4, unlike the other agents, had variable MIC and MLC. The MIC for NaF was 300 ppm and its MLC was 10 fold higher; $SnCl_2$ had a MIC of 200 ppm Cl and MLC of 225 ppm Cl. With respect to tin concentration, SnF_2 had the lowest MIC and MLC, being about 3 and 2 fold more potent in MIC and MLC, respectively, than the other Sn-containing compounds.

Bacterial Acid Production and Growth at Low Fluoride Levels

There were slight effects of the various F^- agents or $SnCl_2$ at 10 ppm on the rate of culture pH fall and generation time (Figures 1 & 2). Slowing of the generation time was most notable in the presence of SnF_2, $SnCl_2$, and Na_2SnF_6, and differences in growth rate from the fluoride-free and tin-free control could not be observed for TiF_4 and NaF. However, the growth yield in the presence of all of the compounds, except TiF_4, was clearly lower than in their absence. Similarly,

NaF, SnF_2, and Na_2SnF_6 slightly retarded the rate of culture pH fall but $SnCl_2$, as well as TiF_4 had no appreciable effect. The terminal pH was not as low for cultures incubated with SnF_2, NaF, or Na_2SnF_6 as for those with $SnCl_2$, TiF_4 or without additive.

DNA and Glucan Analyses

Table 2 presents the ranking of treatment effects for various test agents and water controls with respect to the amount of DNA and alkali soluble glucan (ASG) per unit enamel surfact area, as well as the amount of ASG per DNA.

Less enamel-adherent DNA and alkali soluble glucan (ASG) were found in the presence of SnF_2, Na_2SnF_6, and NaF compared to other compounds tested, with SnF_2 showing the least. However, there was no significant difference in the ratio µg ASG/µg DNA among these samples. This suggests that the lower ASG found in the fluoride test groups was due to the presence of fewer bacteria on the enamel in these groups and that these agents, especially SnF_2, interfered with growth or adhesion of bacteria to the enamel. No water soluble glucan was detected in the enamel-adherent cell mass.

Comparison of "total DNA" at the end of three days growth, i.e., enamel-adherent bacterial DNA and culture liquor DNA from the third day's culture fluid, revealed less DNA/ml medium in the presence of the various fluoride salts and $SnCl_2$ than in the absence (Table 3). The SnF_2-supplemented cultures had the least DNA. However, there were statistically higher ratios of total ASG/DNA (Table 3) for SnF_2, Na_2SnF_6, and NaF than for TiF_4, $SnCl_2$ and the water control groups. ASG derived from adherent and nonadherent organisms, expressed per ml of culture medium, increased in the presence of these agents than in the presence of $SnCl_2$, TiF_4 and water controls. Thus, SnF_2, Na_2SnF_6 and NaF fostered glucan synthesis while inhibiting bacterial growth. The most potent agent with the regard SnF_2, as shown by the ratios of ASG/DNA and WSG/DNA.

Tin Content of Bacteria Adherent to Stainless Steel Wire

As expected, no tin was detected in the three day wire-adherent bacteria in the control, TiF_4, and NaF treatment groups, while the bacteria grown in the presence of SnF_2, $SnCl_2$, and Na_2SnF_6 contained tin. The plaque incubated in SnF_2-supplemented media had more tin/mg plaque than in those specimens cultured in $SnCl_2$-, or Na_2SnF_6-supplemented media (Table 4).

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DISCUSSION

The relatively high bacteriostatic and bactericidal activity observed for SnF_2 at low concentrations cannot be explained by the separate action of stannous or fluoride ions alone, since neither $SnCl_2$ nor NaF had MIC or MLC values nearly as low as SnF_2. The greater potency of SnF_2 than NaF and $SnCl_2$ has been previously observed (Tinanoff et al., 1976; Tinanoff and Camosci, 1980). The mechanism(s) for these differences is suggested by other findings in this study.

Although several experiments showed an effect of stannous ions on S. mutans, the alteration of acid production seems to be due primarily to fluoride because $SnCl_2$ at the levels tested had no detectable effect on the rate of culture pH fall. Inhibition of acid production by salivary and plaque bacteria by less than 1 ppm F has been known for some time (Bibby and Van Kesteren, 1940; Wright and Jenkins, 1954). Furthermore, plaque collected from subjects living in fluoridated areas exhibits less acid production on exposure to sucrose than plaque from subjects living in nonfluoridated areas (Jenkins et al., 1969). These findings may be at least partially explained by the observation that fluoride interferes with enolase, essential for glycolysis and the energetics supporting membrane transport of glucose and sucrose (Hamilton, 1977; Slee and Tanzer, 1979).

Bacterial growth yields also were lower in all media supplemented with either fluoride or tin compounds except for TiF_4 (recall that the level of TiF_4 tested was lower than 10 ppm). The decreased growth rate and yield may be due in part to the altered carbohydrate metabolism of S. mutans in the presence of fluoride, as is suggested by the increased total culture glucan production in its presence. Furthermore, heavy metals, such as tin, are known to have a "germicidal" effect because of their ability to precipitate proteins (Salle, 1968). Therefore, tin may be metabolically disruptive, accounting for the detectably decreased growth rate in its presence, compared with the growth rate in the presence of NaF at the

same low concentration.

There was a decrease in bacterial DNA and glucan attached to enamel specimens exposed to NaF, SnF_2 or Na_2SnF_6, with SnF_2 having the greatest effect. No differences were noted in the amount of enamel-adherent ASG among groups when these data normalized for the variations in bacterial quantity. Consequently, the decreased enamel-adherent alkali soluble glucans (ASG/mm^2) in media supplemented with fluorides may be explained as resulting from either reduction of bacterial adherence to the enamel or reduction in bacterial growth.

Although no differences in enamel-attached glucans due to fluoride or tin were found, an overall increase in "total" water and alkali soluble glucan for those test groups exposed to NaF, SnF_2 and Na_2SnF_6 was observed. The total glucan calculation represents the enamel-attached and unattached cell-associated glucans (alkali soluble glucan) and WSG component in the media. This increase in both alkali- and water-soluble glucan components was most evident in the SnF_2 treatment groups, with 4 times more alkali soluble and 10 times more water soluble glucan being produced in the SnF_2 group as compared to the control.

A difficulty in this experimental design is in the calculation of total DNA because bacterial adherent to the wire were removed for tin analysis. Yet, since the bacterial dry weight used for tin analysis was similar for each group, there was no significant effect on the glucan/DNA ratios computed.

Most studies that have evaluated the effect of fluoride on bacterial extracellular polysaccharide (EPS) production have reported decreased under the influence of fluoride concentrations ranging from 10 to 70 ppm F (Loesche et al., 1973 and 1975; Bowen and Hewitt, 1974). Recently, Treasure and Handelman (1980) verbally reported extracellular polysaccharide synthesis/bacterial protein data for several strains of S. mutans incubated under the

influence of 25 or 50 ppm F. In contrast to the earlier studies, they found increased synthesis under the influence of fluoride, consistent with the present data.

We found greater tin uptake in SnF_2-treated cells than $SnCl_2$-treated ones. Rölla (1976) and Svatun et al. (1977) have suggested that tin ions may compete with calcium for acidic groups on the bacterial surface, thus concentrating this cation on the cell surface. However, increased tin in bacterial cells exposed to SnF_2 could possibly result directly or indirectly from accumulation of fluoride by bacteria (Tinanoff and Camosci, 1980). Fluoride is accumulated by plaque (Jenkins and Edgar, 1969), whereas chloride apparently is not concentrated by bacteria (Mitchell and Moyle, 1959; Schultz et al., 1962). (It should be noted that $SnCl_2$ and SnF_2 solutions were formulated for equimolar Sn concentrations. SnF_2 and Na_2SnF_6 were adjusted for equimolar F concentrations, not Sn concentrations. This may account for the lower tin uptake from Na_2SnF_6 compared to SnF_2).

SnF_2 appears to have the most significant antiplaque properties against S. mutans of those fluoride compounds tested at a concentration of 10 ppm F. The increased effectiveness of SnF_2 appears related to increased cellular tin accumulation.

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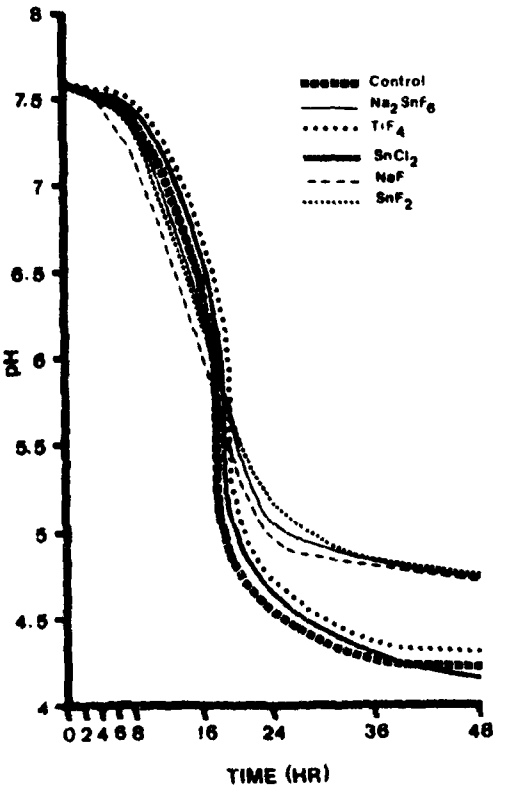

Figure 1: Acid production by S. mutans NCTC 10449S in medium supplemented with 5% sucrose and various fluoride compounds (10 ppm F) or $SnCl_2$ (10 ppm Cl).

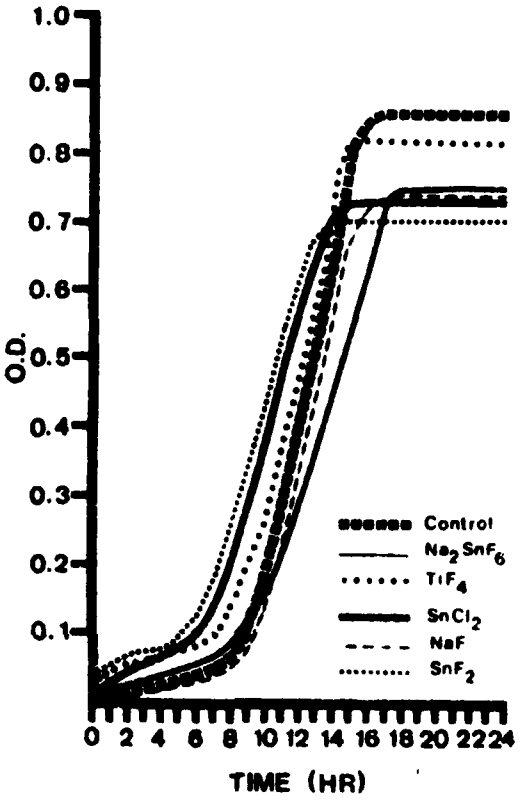

Figure 2: Growth of S. mutans NCTC 10449S in medium supplemented with 5% sucrose and various fluoride compounds (10 ppm F) or $SnCl_2$ (10 ppm Cl).

Test Compound	MIC		MLC	
	ppmF^-	ppmSn	ppmF^-	ppmSn
SnF_2	60	180	125	375
$SnCl_2$	(200 ppmCl^-)	600	(225 ppmCl^-)	675
Na_2SnF_6	600	600	675	675
NaF	300		3000	
TiF_4	550 $\pm$ 25		575 $\pm$ 25	

Table 1: Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of various fluoride compounds and $SnCl_2$ against S. mutans NCTC 10449S.

	μg DNA / mm² Enamel*	Subsets**		μg ASG / mm² Enamel*	Subsets		μg ASG / μg DNA	Subsets
Control	0.17 ± .02		TiF_4	1.35 ± .43		TiF_4	9.02 ± 1.24	
$SnCl_2$	0.15 ± .00		$SnCl_2$	1.19 ± .05		$SnCl_2$	7.42 ± 0.85	
TiF_4	0.14 ± .03		*Control*	1.10 ± .16		NaF	7.18 ± 0.70	
Na_2SnF_6	0.12 ± .02		NaF	0.83 ± .19		SnF_2	6.72 ± 1.28	
NaF	0.11 ± .02		Na_2SnF_6	0.62 ± .04		Control	6.39 ± 0.59	
SnF_2	0.05 ± .00		SnF_2	0.32 ± .09		Na_2SnF_6	5.37 ± 0.95	

* Mean of 3 samples ± S.D.
** Homogeneous subsets using Analysis of Variance with Scheffe procedure ($p \leq .01$)

Table 2: Amount of bacterial DNA and alkali soluble glucan (ASG) adherent to enamel after three days' incubation of S. mutans NCTC 10449S in medium supplemented with various fluoride compounds (10 ppm F), $SnCl_2$, (10 ppm Cl) or H_2O (control).

Total μg DNA/ml *		Subsets**
Control	3.56 ± .41	
NaF	2.87 ± .86	
Na_2SnF_6	2.72 ± .75	
TiF_4	2.66 ± .57	
$SnCl_2$	2.52 ± .20	
SnF_2	1.27 ± .11	

Total μg ASG/ml		Subsets
Na_2SnF_6	58.1 ± 12.0	
NaF	55.2 ± 2.2	
SnF_2	55.1 ± 13.0	
TiF_4	33.0 ± 7.9	
Control	32.9 ± 3.8	
$SnCl_2$	21.7 ± 2.6	

Total μg ASG/μg DNA		Subsets
SnF_2	33.2 ± 3.6	
Na_2SnF_6	23.3 ± 10.7	
NaF	20.2 ± 5.3	
TiF_4	12.4 ± 0.45	
Control	9.3 ± 1.3	
$SnCl_2$	8.6 ± 0.81	

Total μg WSG/ml		Subsets
NaF	57.5 ± 7.8	
SnF_2	56.6 ± 10.5	
Na_2SnF_6	47.2 ± 8.3	
TiF_4	17.7 ± 4.8	
Control	15.5 ± 0.9	
$SnCl_2$	10.9 ± 4.0	

Total μg WSG/μg DNA		Subsets
SnF_2	45.0 ± 11.3	
NaF	21.4 ± 7.2	
Na_2SnF_6	14.1 ± 3.9	
TiF_4	6.7 ± 1.6	
$SnCl_2$	4.2 ± 1.3	
Control	4.0 ± 0.48	

* Mean of 3 samples ± S.D.

** Homogeneous subsets using Analysis of Variance with Scheffe procedure ($p \leq .01$)

Table 3: Total bacterial DNA, alkali soluble glucan (ASG), and water soluble glucan (WSG) adherent to enamel and present in the culture fluid of the third day's incubation of S. mutans NCTC 10449S. Growth medium was supplemented with either fluoride compounds (10 ppm F), $SnCl_2$ (10 ppm Cl), or H_2O (control).

	Plaque Dry Weight (mg)	Sn˚/ Total Sample (ppm)	Sn˚/ mg. plaque (μg)
Control	1.8	N.D.	N.D.
Na_2SnF_6	1.5	6	4
TiF_4	3.0	N.D.	N.D.
$SnCl_2$	2.4	48	20
NaF	1.7	N.D.	N.D.
SnF_2	1.4	47	34

Table 4: Tin content of bacteria harvested from the wires suspending enamel specimens of various F, Sn- or control groups. Samples were pooled, dried, and analyzed for tin using atomic absorption spectrophotometry. The limit of detection of total Sn using this method is< 1 ppm. N.D. - non detected.

The Effect of Fluoride and Stannous Ions on S. Mutans: II. Ultrastructural Changes

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ABSTRACT

Electron microscopy was used to observe the effects of various salts of fluoride and tin on S. mutans NCTC 10449. Increase in extracellular material was noted in those electron micrographs of S. mutans cultured in the presence of fluoride (10 ppm F). Electron lucent holes (polyphosphate), a sign of unbalanced growth, were found in 81% of the bacteria cultured in media containing SnF_2 (10 ppm F) and in 32% of the bacteria cultured in media containing $SnCl_2$ (Sn = Sn in SnF_2). Electron dense granules (tin) were found intracellularly in 23% of the bacteria in the SnF_2 group and in 10% of the bacteria in the $SnCl_2$ group. The intracellular tin accumulation and polyphosphate found in those bacteria exposed to SnF_2 may be distinguishing ultrastructural features of stannous fluoride interaction with certain oral microorganisms.

INTRODUCTION

SnF_2 has been shown to reduce the number of bacteria attached to tooth enamel (for review see Tinanoff and Weeks, 1979). Even though this antiplaque mechanism is not completely elucidated, electron microscopy has shown separation among bacteria, and between bacteria and enamel in those plaques exposed to SnF_2. This finding gave initial consideration that either the charge on the bacteria may have been altered, or the bacteria were no longer producing the extracellular binding material due to this agent (Tinanoff, Brady and Gross, 1976). More recently, bacterial growth on enamel in vitro was used to examine the effect of plaque formation of a 1 minute, twice daily exposure of various fluoride solutions (250 ppm F). Electron microscopy of the adherent S. mutans exposed to SnF_2 again showed bacterial separation from the enamel. The SnF_2 treated bacteria, as well, were noted to have numerous intracellular electron lucent holes, a finding compatible with unbalanced bacterial growth. Besides the holes in the cells, electron dense granules, identified as tin deposits by electron microprobe, were frequently found associated with these cells (Tinanoff and Camosci, 1980).

The purpose of this investigation was to observe and to quantitate the ultrastructural findings associated with growth of S. mutans in media supplemented with low concentration (10 ppm) of various fluoride or tin containing compounds and to compare these findings to a parallel study examining S. mutans metabolism with the same growth conditions (Ferretti, Tanzer, Tinanoff, 1981).

MATERIALS AND METHODS

Microorganisms and Growth Media

Fresh aqueous solutions of NaF, SnF_2, Na_2SnF_6 and TiF_4 were first prepared at 100 ppm F and then added to Jordan's medium (Jordan, et al., 1960) supplemented with 5% sucrose to produce a fluoride concentration of 10 ppm. $SnCl_2$, equimolar to Sn in SnF_2, served as a control for tin. Deionized water added at the same

volume as the other aqueous solutions served as a F-free, Sn-free control. The final pH of the supplemented Jordan's media in all cases was 7.6 (Ferretti, Tanzer, Tinanoff, 1981). Stainless steel wires (0.036") were suspended into tubes containing Jordan's medium supplemented with the various fluoride compounds or controls. Stock cultures of Streptococcus mutans NCTC 10449S (Tanzer, et al., 1976), maintained in fluid thioglycolate medium, were adapted prior to the experiments to Jordan's medium supplemented with 5% sucrose. Each tube was then inoculated with 0.1 ml of the adapted culture and incubated microaerophillicly at 37°C. The wires with adherent microorganisms were transferred after 24 hours to the appropriate fresh media.

Electron Microscopy

After three day incubation period, the wires with attached microorganisms were fixed in 2.5% gluteraldehyde in phosphate buffer (pH 7.4, 390 mOsm). The fixed microorganisms were mechanically dislodged from the stainless steel wires, postfixed in 1% osmium tetroxide in veronal buffer (pH 7.3) (Warshawsky and Moore, 1967), and washed in phosphate buffer. The specimens were dehydrated in acetone and embedded in Spurr's epoxy resin (Spurr, 1969).

After polymerization of the specimen at 70°C for 24 hours, thin sections of each specimen for electron microscopic observation were cut with a Sorval MT2B ultramicrotome (Sorvall Company, Norwalk, CT) using a diamond knife. Silver-gold colored sections, either unstained or stained with aqueous uranyl acetate followed by lead citrate (Venable and Coggeshall, 1965) were examined at 80 KV with a Zeiss EM 10 electron microscope.

Two representative electromicrographs from each sample at a standard magnification (5,000 x) were used to semi-quantitate certain observations. This procedure entailed counting the number of intracellular and extracellular electron dense granules and the number of electron lucent holes on the selected micrographs

from each specimen. The percent of these structures to the bacteria present in the micrographs was then determined by dividing either the electron lucent holes or the electron dense granules by the total number of bacteria found on the two micrographs.

RESULTS

Increases in extracellular material were apparent in those S. mutans specimens cultured in the presence of NaF, Na_2SnF_6 and SnF_2 as compared to the $SnCl_2$ or water control (for example, Figs. 2 and 4). This increased density of extracellular material was not; however, present in the TiF_4 group, presumably due to the low levels of fluoride in the growth media with this agent, i.e., the nominal 10 ppm F level for the TiF_4 solution was actually only 2.3 ppm as measured by fluoride electrode, (Ferretti, Tanzer, Tinanoff, 1981).

An altered cell appearance in those bacteria incubated with either $SnCl_2$ or SnF_2 was evident with the most of the atypical coccal morphology found in the SnF_2 group, (Fig. 4). Electron-lucent holes (bacterial polyphosphate) were found infrequently in those bacteria in the water control, Na_2SnF_6, TiF_4 and in the NaF group. However, these holes were present more frequently in bacteria in the $SnCl_2$ group and in the majority of the bacteria in the SnF_2 group (Table 1). Quantition of the electron dense granules (tin) revealed the bacteria in the SnF_2 and $SnCl_2$ groups had granules associated with the cell boundaries; yet there was a gr ater percentage of bacteria in the SnF_2 group that had intracellular granules (Table 1).

DISCUSSION

Further information on the mechanism by which fluoride and tin at low concentrations affect S. mutans may be derived from the findings in the present study. Increase in extracellular material was observed in electron micrographs of bacteria grown in the fluoride supplemented media. Our parallel metabolism study also suggests alteration in glucan production due to low levels of fluoride.

The electron lucent holes were noted in the bacteria in all the test groups, but most frequently found in those bacteria exposed to SnF_2 and $SnCl_2$. Such holes are compatible with the artifact which is seen when bacterial polyphosphate is

examined under the electron microscope (Voelz, et al., 1966). Polyphosphates have been identified in a variety of microorganisms (Harold, 1966) including S. mutans (Tanzer and Kirchevsky, 1966; Tinanoff and Tanzer, 1979: Tinanoff and Camosci, 1980). This highly anionic phosphate is believed found in cells when nutritional conditions are not favorable to growth (Harold, 1966). The holes found in bacteria cultured with $SnCl_2$ and especially those cultured with SnF_2 may indicate unbalanced growth (Tinanoff and Camosci, 1980). This finding confirms the altered growth patterns of those bacteria in the presence of tin noted in the parallel study (Ferretti, Tanzer, Tinanoff, 1980).

Electron dense granules observed intracellularly and on cell boundaries of those S. mutans exposed to $SnCl_2$ or SnF_2 have been previously noted and identified as tin by electron microprobe (Tinanoff and Camosci, 1980). The presence of greater numbers of intracellular tin granules in these organisms exposed to SnF_2 compared to those exposed to $SnCl_2$ is compatible with a hypothesis that tin enters the cell coupled to fluoride (Tinanoff and Camosci, 1980). This semiquantitative finding appears to correlate well with atomic absorption results in which bacteria exposed to $SnCl_2$ had less Sn/mg plaque than bacteria exposed to SnF_2 (Ferretti, Tanzer, Tinanoff, 1981). Besides the previously noted alteration of bacterial attachment due to SnF_2, this study suggests that SnF_2 produces metabolic alterations as a result of the intracellular tin accumulation.

Table 1: Percentage of bacteria, counted on electron micrographs, containing electron-lucent holes (polyphosphate), or electon dense granules (tin). Bacteria grown for three days in complex media supplemented with various fluoride compounds or controls.

Growth Media Supplement	Total # of Bacteria Counted	Electrolucent-Holes	Electron Dense Granules		
			Cell Boundary	Intracellular	Total
H_2O	937	12%			
NaF (10 ppm F)	754	21%			
TiF_4 (2.3 ppm F)	543	13%			
Na_2SnF_6 (10 ppm F)	848	12%			
$SnCl_2$ (Sn=Sn in SnF_2)	987	32%	6%	10%	16%
SnF_2 (10 ppm F)	643	81%	4%	23%	27%

FIGURE LEGENDS

Figure 1: Transmission electron micrographs of S. mutans NCTC 10449S incubated for 3 days in Jordan's medium supplemented with 5% sucrose (F-free, Sn-free control). Coccal bacteria in an extracellular matrix is evident at low magnification. Electron lucent holes (polyphosphate) is found in a few cells (arrow) x 11,000 uranyl acetate, lead citrate. High magnification inset demonstrates typical gram positive cocci with associated extracellular material x 55,000, uranyl acetate; lead citrate.

Figure 2: T.E.M. of S. mutans incubated for 3 days in Jordan's medium containing 5% sucrose and NaF (10 ppm F). More extracellular material is apparent than in the control photomicrograph (Figure 1) at low magnification x 11,000 uranyl acetate, lead citrate. High magnification x 55,000 uranyl acetate;lead citrate.

Figure 3: T.E.M. of S. mutans incubated for 3 days in Jordan's medium containing 5% sucrose and $SnCl_2$ (Sn=Sn in SnF_2). More intracellular electronlucent holes are evident at low magnification (arrow) than in controls x 11,000, no stain. High magnification reveals electron dense granules, in this case, intracellular and on the cell boundary (black arrows) x 55,000, no stains.

Figure 4: T.E.M. of S. mutans incubated for 3 days in Jordan's medium containing 5% sucrose and SnF_2 (10 ppm F). Note the presence of numerous electron lucent holes (white arrows) and apparently distorted cell shapes at low magnification x 11,000, no stains. High magnification inset shows electron dense granules in the cells and on the cell walls (black arrows), x 55,000.

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